GEOMAR, Helmholtz Centre for Ocean Research

Master Thesis

The trophic ecology of the oceanic orangeback squid *Sthenoteuthis pteropus* (Steenstrup) in the eastern tropical Atlantic

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Index of abbreviations and symbols

‰	per mil
%	percent
µm	micrometer
ANOSIM	analysis of similarity
ANOVA	analysis of variance
cm	centimeter
FO	frequency of occurrence
FO%	frequency of occurrence given in percent
g	gram
GAM	generalized additive model
m	meter
ML	mantle length
N	number
N%	number given in percent
Ref	Reference Station
RV	research vessel
S	Senghor Seamount
SFI	stomach fullness index
SIMPER	similarity percentage routine

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Abstract

In the eastern tropical Atlantic, the orangeback flying squid *Sthenoteuthis pteropus* is an opportunistic short-living carnivore and among the fastest growing squids. It is one of the dominant members of the epipelagic nekton community. Due to its high plasticity and ability to tolerate environments with low oxygen concentrations it is supposed to better cope with a changing ocean than many other nekton organisms. So far our understanding of its trophic ecology is limited. The present study characterises the role of *S. pteropus* in the pelagic food web of the eastern tropical Atlantic by investigating its diet and how its feeding habits change during ontogeny and geographical distribution.

During hand jigging 129 specimens of *Sthenoteuthis pteropus*, ranging from 15.5 to 47.5 cm (dorsal mantle length), were caught in the eastern tropical Atlantic in the course of three oceanic expeditions in 2015. Besides body mass and size, sex, maturity stages and stomach fullness were recorded. Stomach content analysis was combined with stable isotope analysis of muscle tissue ($\partial^{15}N$ and $\partial^{13}C$) to describe diet and current trophic position of *S. pteropus*. Stable isotope analysis of the gladius, the chitinous backbone of the squid, which acts as an archival structure, were also conducted to study fine-scale variation in squid trophic ecology and foraging migration.

This study demonstrates that *Sthenoteuthis pteropus* mainly preys on myctophid fishes, but also on cephalopods and crustaceans. It shows a highly opportunistic and variable feeding behaviour and potentially competes with large predatory fishes for the same prey resource. The trophic position of *S. pteropus* increases by likely one trophic level with increasing mantle length. The reconstructed feeding chronologies of the gladii reveal high intra- and inter-individual variability in the squid's trophic position and foraging area that cannot be recognized in the squid diet and muscle tissue. This complex life history strategy suggests a high plasticity and capability of *S. pteropus* to rapidly colonize new areas and niches made available and to adapt to new environmental conditions.

1 Introduction

1.1 Oceanic squids

Cephalopods are molluscs that include nautiluses, vampire squids, octopods, cuttlefish and squids. In this study we are focusing on oceanic squids. They are active, fast moving predators and feed voraciously and with great versatility on a variety of prey (Macy 1982, Collins et al. 1994, Quetglas et al. 1999, Markaida 2006, Ruiz-Cooley et al. 2006, Bazzino et al. 2010, Hunsicker et al. 2010) to maintain a high metabolism (Seibel et al. 1997, Shulman et al. 2002) and increased growth rates (Wells & Clarke 1996). Squids generally have a lifespan of about one year and are semelparous, that means they only have one reproductive cycle (Boyle & Rodhouse 2005).

Oceanic squids play a key role in the trophic structure of marine pelagic ecosystems (Clarke 1996a, Piatkowski et al. 2001) which is underlined by their large biomass (Doubleday et al. 2016) and importance in the diet of marine predators such as fishes and marine mammals (Clarke 1996b, Smale 1996).

Short-term studies expect active squids to be negatively affected by decreasing oxygen concentrations under ocean warming, expanding oxygen minimum zones and acidification due to their high metabolism and energy demand (Pimentel et al. 2012, Rosa et al. 2012, Kaplan et al. 2013, Spady et al. 2014, Seibel 2016). However, in the long term they may respond differently to climate change. Hatfield (2000) provided evidence that the Patagonian squid *Loligo gahi* accelerated growth remarkably due to increased temperatures during the squid's early growth period, leading to significant differences in size-at-age for adult squid. Another prominent example for the change in cephalopod dynamics is *Dosidicus gigas*, the Humboldt squid. *D. gigas* inhabits the eastern Pacific Ocean and has substantially expanded its geographical range during climate-related changes in oceanographic conditions (Stewart et al. 2014) and the decline in tuna and billfish populations in this area. This range expansion also coincides with a decline in Pacific hake abundance, the most important commercially fished groundfish species off western North America and prey of *D. gigas* (Field et al. 2007, Zeidberg & Robison 2007). Furthermore, *D. gigas* switched size-at-maturity as a response to an El Niño event in 2009 – 2010, showed avoiding behavior of unfavorable environments (Hoving et al. 2013) and changes in feeding behavior due to environmental

variation (Field et al. 2007). This illustrates that oceanic squids are capable of taking over niches of overexploited fishes and due to their plastic physiology and life history are able to rapidly adapt to environmental changes. There is growing evidence that cephalopods are among the few marine organisms expanding in response to a changing environment (Caddy & Rodhouse 1998, Vecchione et al. 2009, Hoving et al. 2013, Doubleday et al. 2016). Over the last six decades, cephalopod abundance has increased globally due to a combination of finfish overfishing and climate change (Doubleday et al. 2016). The potential impacts of these changes on marine food webs are challenging to predict, and the ecological role of fast-growing and high adaptive squids needs to be examined in more detail. Several studies suggest, that an increasing abundance of cephalopods could impact many prey species including commercially valuable fishes (Field et al. 2007, Zeidberg & Robison 2007, Stewart et al. 2014). However, marine top predators and commercial fisheries could benefit from increasing cephalopod populations (Doubleday et al. 2016). Their extreme flexibility in ecology and life history could allow oceanic squids to cope with climate change (Pecl & Jackson 2008, Hoving et al. 2013, Doubleday et al. 2016).

Another aspect to consider with respect to alterations in trophic structure is fishery activity. Due to overfishing of finfish stocks and the rapid development of modern fishing techniques, cephalopod yields have increased from about 1 million metric tonnes in 1970 to more than 4 million metric tonnes in 2007 and the number of cephalopod species entering commercial fisheries also increased significantly since 1984 (Roper et al. 2010). Understanding the potential impacts of fisheries-induced alterations to trophic structures and climate change on the importance of squids in the food web is essential to fill the gaps in our understanding of their ecology.

1.2 The orangeback flying squid *Sthenoteuthis pteropus*

This study focuses on a related species of *Dosidicus gigas:* the orangeback flying squid *Sthenoteuthis pteropus*. Like *Dosidicus gigas* this species belongs to the family Ommastrephidae. Ommastrephid squids are widely distributed in all major oceans and feed mainly on fishes, particularly myctophids, as well as on cephalopods and crustaceans (Piatkowski et al. 1998, Lordan et al. 1998, Markaida 2006, Parry 2006, Dunn 2009). Several

studies show a systematic increase in trophic level through ontogeny (Ruiz-Cooley et al. 2006, 2010, Cherel et al. 2009).

S. pteropus is an opportunistic short-living carnivore and among the fastest growing squids (Arkhipkin & Mikheev 1992). It undertakes diel vertical migrations from surface layers at night for active feeding down to 1200 m during the day (Zuyev & Nikolsky 1993) and is one of the dominant members of the epipelagic nekton community in the eastern tropical Atlantic (Zuyev et al. 2002) (Fig. 1 left). This area is characterized by a pronounced oxygen minimum zone (Stramma et al. 2008) (Fig. 1 right) which is expanding due to global warming and eutrophication (Stramma et al. 2010). *D. gigas* and *S. pteropus* are adapted to temporarily live and actively forage in environments with low oxygen concentrations by metabolic suppression (Rosa & Seibel 2010), anaerobic metabolism (Shulman et al. 2002) and active migration (Zuyev et al. 2002, Gilly et al. 2006, 2010, Stewart et al. 2013, Seibel 2016).

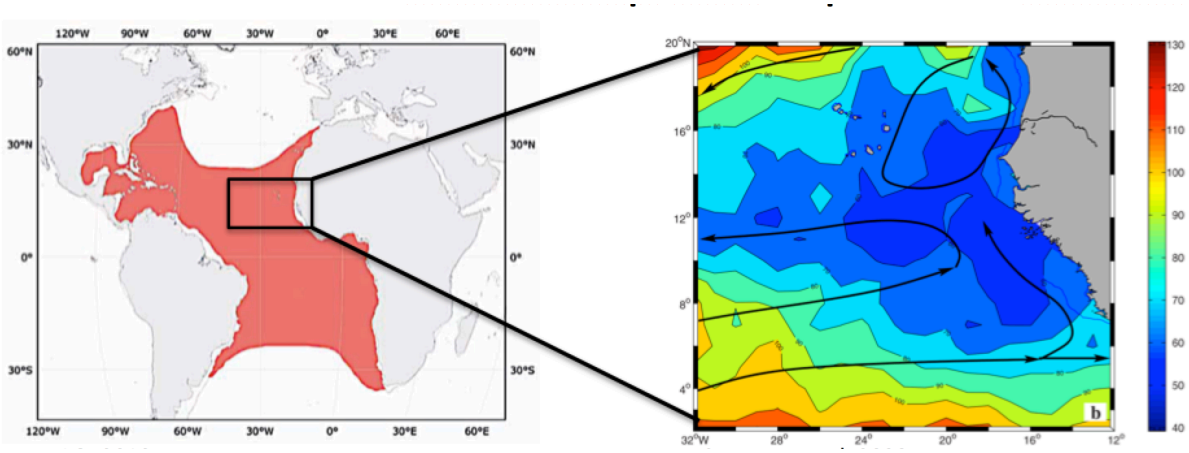

Figure 1. Left: Distribution of *Sthenoteuthis pteropus* (after Roper et al. 2010); right: oxygen distribution in µmol kg^{-1} in the eastern tropical Atlantic at 400 m depth showing minimum oxygen values around Cape Verde (after Stramma et al. 2008)

The complex population structure of *S. pteropus* consists of a small early-maturing form and a large late-maturing form. The life cycle of all female squid is about one year independent of their size (Zuyev & Nikolsky 1993), therefore early and late maturing refers to slowly and quickly growing. Squid of the small (early-maturing) form reach maturity with 12 – 20 cm and 16 – 35 cm mantle length in males and females, respectively. The large late-maturing form reaches maturity with 18 -28 cm mantle length in males and 30 – 65 cm mantle length in females. Small early-maturing squid are only found in the equatorial zone, but representatives of the large form are distributed throughout the species range. Both forms

are divided into several populations separated by areas with low productivity and oceanographic barriers. They also differ genetically and have been distinguished into a northern, equatorial and southern group in the eastern Atlantic based on the frequency of phenotypes of estherases of squid mantle and skin (Zuyev & Nikolsky 1993, Zuyev et al. 2002).

The most comprehensive study on biology and ecology of *Sthenoteuthis* was published by Zuyev et al. (2002). It emphasizes that *Sthenoteuthis pteropus* is a key component in the food web of the eastern tropical Atlantic. However, investigations of the squid's trophic ecology and ontogeny in a changing environment are still lacking. In order to achieve a better understanding of the ecological role and the trophic position of this species, traditional methods need to be combined with modern techniques, e.g. stable isotope analysis.

Sthenoteuthis pteropus has not been fished commercially yet, but represents a potential fishery resource in the Atlantic Ocean with an instantaneous biomass of 4.2 to 6.5 million tonnes and an annual total biomass production of estimated 34 to 52 million tonnes (Roper et al. 2010). In comparison, the instantaneous biomass of *Dosidicus gigas*, which makes up the world largest cephalopod fishery, is estimated to be 7 million to 10 million tonnes, with catches close to 700 000 tonnes in 2007. This species alone accounted for about 21% of the total squid world catch in 2007 (Roper et al. 2010).

Table 1. Changes of main prey composition of the squid *Sthenoteuthis pteropus* during ontogeny (modified from Zuyev et al. 2002)

Ontogenetic stage	Size	Main food	Trophic position (average)
Paralarvae	0.1 – 0.8 cm	micro- and mesozooplankton	II
Postlarvae	1.0 – 2.5 cm	mesoplanktonic crustaceans	II – III (II)
early Juveniles	3 – 9 cm	macroplanktonic crustaceans	II – IV (III)
late Juveniles	9 – 15 cm	micronektonic fishes	III – V (IV)
Adult, middle-sized	15 – 35 cm	micronektonic fishes	IV – V (IV)
Adult, large-sized	35 – 65 cm	nektonic fishes and squids	IV – V (V)

Like in other ommastrephid squids (Ruiz-Cooley et al. 2004, 2010), the prey spectrum of *S. pteropus* changes during ontogeny (Table 1). Information on its diet is very general and almost no species are mentioned (Zuyev et al. 2002). In early juveniles (3 – 9 cm mantle length (ML)) the diet is dominated by meso- and macroplanktonic invertebrates, mainly copepods, amphipods and euphausiids. The species spectrum of late juveniles (9 – 15 cm ML) and adult middle-sized squid (15 – 35 cm ML) shifts to micronektonic fishes and finally adult large-sized squid (35 – 65 cm ML) mainly feed on nektonic fishes and squids. Six ontogenetic stages can be defined in *S. pteropus*: planktonic paralarvae (0.1 – 0.8 cm), postlarvae (1 – 2.5 cm), early juveniles (3 – 9 cm), late juveniles (9 – 15 cm), nektonic middle-sized adults (15 – 35 cm) and large-sized adults (35 – 65 cm) with two major shifts in diet during ontogeny (Arkhipkin & Mikheev 1992, Zuyev et al. 2002)(Fig. 2).

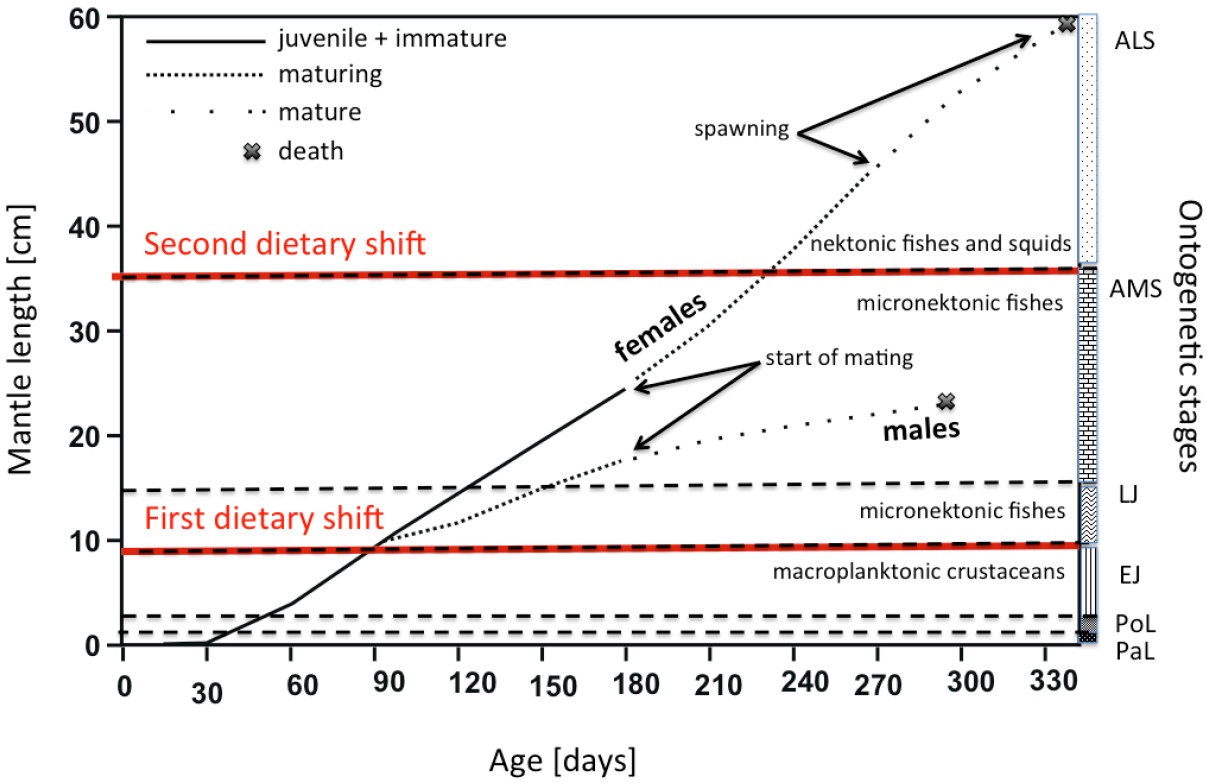

Figure 2. Scheme of life cycle of *Sthenoteuthis pteropus* (modified from Arkhipkin and Mikheev 1992) with dietary shifts (Zuyev et al. 2002). Pal = Paralarvae, PoL = Postlarvae, EJ = early juveniles, LJ = late juveniles, AMS = adult, middle-sized, ALS = adult, large-sized

Sthenoteuthis pteropus is an important prey for marine predators such as swordfish, marine mammals and sharks (Hernandez-Garcia 1995, Zuyev et al. 2002, Vaske Júnior et al. 2004, Cherel et al. 2007) and conversely is an active predator with a wide food spectrum (Zuyev et al. 2002). This combination of bottom-up and top-down effect combined with the high abundance and reproduction rate of these squid could lead to unpredictable responses in the ecosystem if its biomass is altered (Griffiths et al. 2010).

1.3 Approach

This study investigates the general diet, trophic position and foraging patterns of *Sthenoteuthis pteropus* in order to determine its current role in the food web of the eastern tropical Atlantic. We combined traditional stomach content analysis with modern stable isotope analysis.

Stomach content analysis is the traditional method to study the diet of cephalopods (Laptikhovsky 2002, Markaida & Sosa-Nishizaki 2003, Phillips et al. 2003, Markaida 2006). This technique allows prey identification at the species level, using hard parts that are resistant to digestion such as squid beaks, fish otoliths and crustacean exosceletons (Jackson et al. 2007). A problem in analyzing stomach contents in squids is the location of the oesophagus, which passes through the brain. Although cephalopods are capable of capturing relative large prey items their beaks have to bite off small pieces of tissue to swallow and to not injure the oesophagus. Hard parts are therefore often rejected. This selective rejection behavior could bias data on prey species composition and size (Rodhouse & Nigmatullin 1996). Furthermore, stomach contents just represent the last feeding event and do not provide information about long-term dietary habits, as squid have a very fast digestion rate of around 8 – 10 hours in middle-sized squid (Zuyev et al. 2002), (Jackson et al. 2007). This study attempted to overcome those challenges by combining multiple pathways to characterize the trophic role of *Sthenoteuthis pteropus*. Classical stomach content analysis was used to identify prey taxa and to determine how prey composition changes during ontogeny. Since stomach contents do not provide sufficient information about squids trophic position we also analyzed stable isotopes that provide estimates of the long-term trophic position of an animal (Hunsicker et al. 2010, Lorrain et al. 2011).

Stable isotope ratios of carbon (^{13}C / ^{12}C, $\partial^{13}C$) depict the animal's foraging area, habitat and migration patterns. In the marine environment, $\partial^{13}C$ varies little along the food chain, but it reflects spatial variations of the environment and can indicate inshore versus offshore, pelagic versus benthic feeding or latitudinal variations in foraging habitat (Fig. 3) (Takai et al. 2000, Cherel & Hobson 2005, Graham et al. 2010).

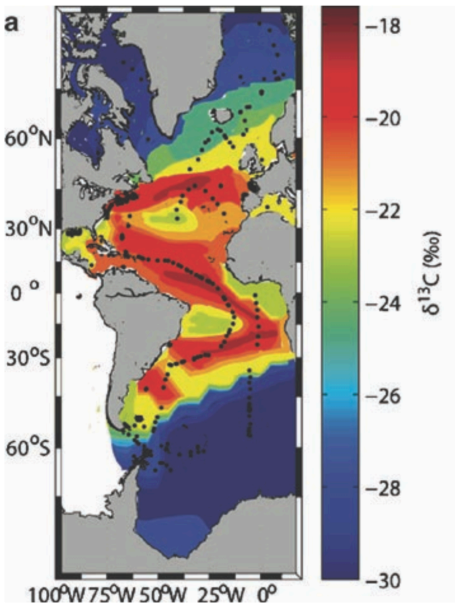

Figure 3. $\partial^{13}C$ isotope values of plankton in the Atlantic Ocean (0–500 m; n = 425). *Black dots* indicate sample locations (figure from Graham et al. 2010)

On the other hand, tissue of consumers are enriched in ^{15}N relative to their food and therefore $\partial^{15}N$ values ($^{15}N/^{14}N$) are indicators of a consumer's trophic position (Cabana & Rasmussen 1996, McCutchan et al. 2003, Vanderklift & Ponsard 2003, Richert et al. 2015). Stable isotope ratios can be measured on hard and soft tissue.

Isotope ratios from soft tissue such as muscle provide time-integrated information about the trophic position of the recently assimilated diet (< 2 months) (Ruiz-Cooley et al. 2013).

To investigate long-term trophic ecology at both the population and individual level, stable isotope analysis of hard tissues like cephalopod beaks, eye lenses and gladii that have no metabolic turnover rate after synthesis are used to trace pathways of organic matter among organisms (Kelly 2000, Ruiz-Cooley et al. 2006, Graham et al. 2010, Hunsicker et al. 2010, Lorrain et al. 2011, Hoving et al. 2014).

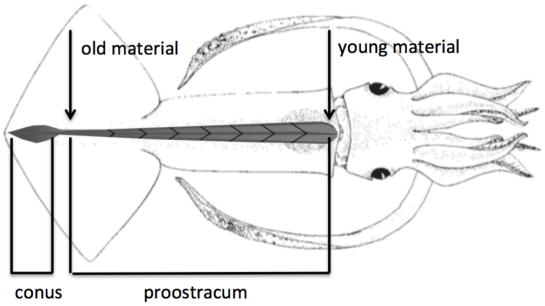

Figure 4. Gladius of a squid separated into a conus and proostracum section (adapted from Roper et al. 2010, Lorrain et al. 2011)

Stable isotope ratios along the gladius of squids (Fig. 4), their internal chitinous shell, have been measured to track ontogenetic shifts and to give a chronological dietary record over their lifetime (Cherel et al. 2009, Ruiz-Cooley et al. 2010, Lorrain et al. 2011). The gladius consists of chitin and proteins, is present in the hatchling and grows continuously by adding chitin to the anterior side, with no metabolic turnover after synthesis. Therefore the gladius is an archival structure (Bizikov 1991, Perez et al. 1996). Investigating feeding chronologies on hard parts is a valuable tool to explore the trophic ontogeny of squid life stages that are difficult to capture, and fewer samples are needed than for stomach content or muscle tissue stable isotope analysis.

1.4 Research questions

This study will investigate the diet and current position of *Sthenoteuthis pteropus* in the pelagic food web of the eastern tropical Atlantic as well as ontogenetic change, individual specialization in feeding habits, foraging habitats and migration. The following research questions will be addressed:

1. What is the ecological role and general diet of *Sthenoteuthis pteropus* in the food web of the eastern tropical Atlantic?
2. Are there differences in prey composition between three research cruises?
3. Does prey composition of *Sthenoteuthis pteropus* shift during ontogeny?
4. Does *Sthenoteuthis pteropus* exhibit ontogenetic shifts and intraspecific variation in trophic position and foraging area?
5. How is the current trophic position of *Sthenoteuthis pteropus* compared to the study of Zuyev et al. (2002)?

2 Materials and Methods

Orangeback flying squid (n = 129) were collected at night by hand jigging in the Cape Verde area of the eastern tropical Atlantic between 17°N – 2°N and 26°W – 21°W in May – June with the research vessel (RV) Meteor (M116), in September – December 2015 with the RV Meteor (M119) and RV Maria S. Merian (MSM49) (Fig. 5, appendix table 6). Specimens from cruise M116 were immediately frozen onboard by -40°C and defrosted in the lab for analysis. Specimens from the cruise M119 were processed on board (see below) and the stomach contents were stored in 70% ethanol. Specimens from the cruise MSM49 were also processed on board and the stomachs were stored in -40°C. Mantle length (to the nearest millimeter) and body weight (to the nearest gram) were measured in all squid. Sex and maturity stage were determined according to Lipiński & Underhill (1995). A qualitative, visual stomach fullness index (SFI) was assigned: 0, empty; 1, traces of food; 2, filled less than half; 3, filled more than half; 4, full; 5, exclusively crammed, its walls distended (Breiby & Jobling 1985). Samples of muscle tissues from individuals of the cruise MSM49 were stored in -80°C. Stomachs and gladii from individuals of the cruise MSM49 and M116 were kept frozen at -40°C for further analysis.

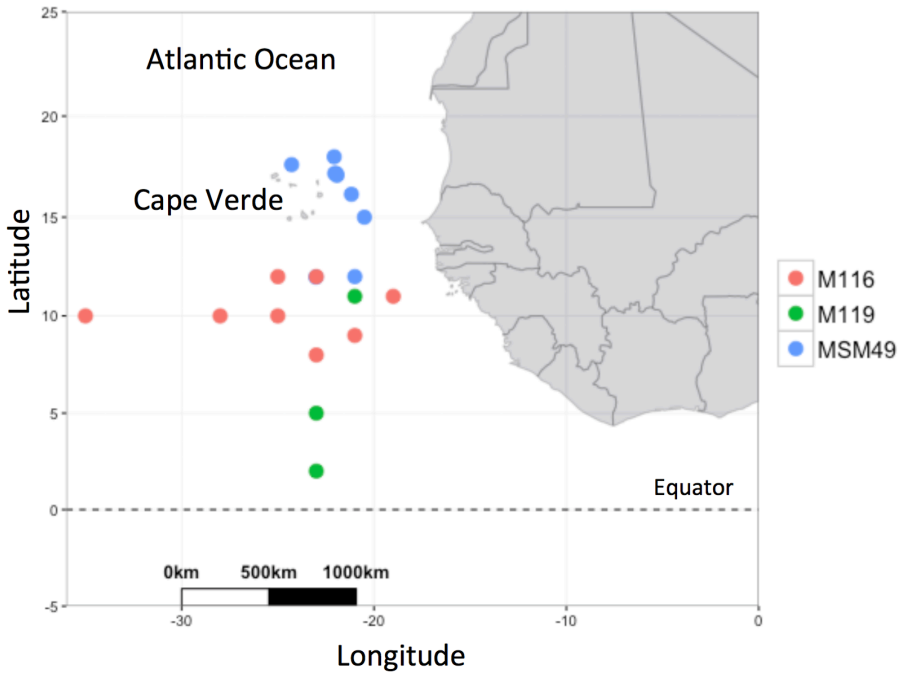

Figure 5. Capture locations of *Sthenoteuthis pteropus* in the tropical eastern Atlantic in 2015 during three research cruises. The different colours depict the different research cruises (red = M116; green = M119; blue = MSM49).

2.1 Stomach content analysis

Stomachs were collected from all 129 orange-back flying squid ranging from 15.2 – 47.5 cm mantle length. Stomach contents were screened through a 300 µm mesh sieve in order to retain prey items useful for identification. Prey items were identified to the lowest possible taxon under a binocular microscope (0.85x). Fish sagittal otoliths were identified according to Smale et al. (1995), Tuset et al. (2008), Campana (2004) and the consultation of Dr. Werner Schwarzhans. Cephalopod beaks were identified using the identification key of Clarke (1986) and the consultation of Dr. Uwe Piatkowski and crustaceans were identified by their exosceletons and the help of Prof. Dr. Stefanie Ismar.

Frequency of occurrence and number were used to quantify the diet (Cailliet 1977, Markaida 2006). The number of fishes or cephalopods consumed was estimated as the maximum counting of right or left fish otoliths or of upper or lower cephalopod beaks (Antonelis et al. 1984). Frequency of occurrence (%FO) was calculated as the percentage of *Sthenoteuthis pteropus* that fed on a certain prey, and number (%N) is the number of individuals of a certain prey relative to the total number of individual prey.

2.2 Stable isotope analysis

Stable isotope analysis was only conducted on the individuals caught in December 2015 (MSM49). Gladii from the 5 largest individuals (> 40.0 cm ML, all females), one male individual (20 cm ML) and muscle tissue from all 54 individuals (18.4 – 47.5 cm ML, females = 44; males = 10) were immediately frozen after capture at -20°C and -80°C, respectively.

The gladius is a long chitinous structure that grows with the animal and is therefore an archival structure. It grows mainly by accretion of chitinous material to the proximal end of the proostracum (near the head) (Fig. 4). To a lesser extent, newly formed material is added to the posterior side of the conus as well leading to an asymmetrical growth both towards the head and to the end of the fins at different rates. Only the proostracum was used for stable isotope analysis (Ruiz-Cooley et al. 2010, Lorrain et al. 2011), because its growth increments are clearly distinguishable and represent the lifespan of a squid. Near the conus region, the proostracum becomes fainter as the gladius narrows posteriorly and cannot be distinguished anymore (Bizikov 1991; Perez et al. 1996). Samples taken at the anterior part of the proostracum therefore depict the youngest material and most recent stable isotopes,

while samples taken at the distal end of the proostracum correspond to older material when the animal was young.

Gladii were cleaned with distilled water, dried with KimWipes (Kimberly-Clark), measured to the nearest mm and the proostracum was cut in 1 or 2 cm increments following the V shape of the growth lines. The gladius samples ($n_{subsamples}$=135) were freeze-dried for 24h, ground into fine powder with mortar and pestle and freeze-dried again for 4h. The samples were weighed with a microbalance and 80 – 120 µg of the powder were transferred into tin containers for isotopic analysis. To estimate the current trophic position of the squid in the food web and to control for differences between muscle and gladius stable isotope ratios, stable isotopes ($\partial^{13}C$; $\partial^{15}N$) were measured from samples of mantle muscle tissue of 54 squid. Prior to analysis muscle tissues were freeze-dried for 24h, ground to a fine powder, lipids were extracted using chloroform and the samples were dried again over night at 50°C. Gladius samples were not acidified because chitinous structures like beaks or gladius do not contain significant amounts of lipids, that could bias ^{13}C values (Jackson et al. 2007). Additionally, the gladius and lipid-free muscle tissue of one male (21 cm) was analyzed to compare with the larger ones. Its gladius was cut into 2 cm consecutive sections following the procedure of the large squid.

Because of the large amounts of chitin in the gladii, it is depleted in $\partial^{15}N$ when compared to muscles, therefore resulting in lower nitrogen isotope values (DeNiro & Epstein 1981, Webb et al. 1998). For the gladii, we conducted lipid corrections based on the method of Post et al. (2007) on $\partial^{13}C$ values because C/N ratios were higher than 3.5. This method led to a small shift in $\partial^{13}C$ by 1‰ in average and very similar patterns over time compared to the original values (appendix Fig. 20). $\partial^{13}C$ muscle isotope values were not corrected for lipid content, because lipids have been extracted beforehand.

Isotope ratios of C and N were measured using an elemental analyzer system (NA 1110, Thermo, Milan, Italy) connected to a temperature-controlled gas chromatography oven (SRI 9300, SRI Instruments, Torrance, CA, USA), which contained a column for permanent gases. Separated sample gases and the reference gases N_2 and CO_2 were transferred via a ConFloIII^{TM} interface (Thermo Fisher Scientific, Bremen, Germany) to the isotope ratio mass spectrometer (Delta^{Plus} Advantage, Thermo Fisher Scientific). The measured isotope ratios are given as ∂ values in per mil deviation (‰) from the standard reference material Vienna

PeeDee Belemnite (VPDB) and atmospheric nitrogen following the equation $\partial X = [(R_{Sample}/R_{Standard})-1]x1000$ where X refers to ^{13}C or ^{15}N and R represents the ratio of the heavy isotope to the light isotope ($^{13}C/^{12}C$ or $^{15}N/^{14}N$). Laboratory gas cylinders of CO_2 and N_2 were used as working standards and calibrated against primary solid standards (IAEA-N1, -N2, -N3, USGS24, NBS22). The lab standard acetanilide used to estimate C and N content for each sample series was measured every 7 sample and had a standard deviation of +/-0.16‰ for $\partial^{15}N$ and +/-0.39‰ for $\partial^{13}C$.

2.3 Data analysis

An Analysis of Similarity (ANOSIM, Clarke 1993) was deployed to identify differences in prey composition between the three research cruises. A similarity percentage routine (SIMPER, Clarke 1993) was applied to examine which prey species were most responsible for differences between the cruises. The number of all species were fourth-root transformed to smoothen the dominating effect of the most abundant species (Field et al. 1982). Additionally, various univariate indices were calculated to test for differences in species diversity and evenness (Table 2). ANOSIM, SIMPER and the univariate indices were conducted with the software PRIMER v6 (Clarke & Gorley 2006).

Table 2. Univariate indices to test for differences in species diversity and evenness

Univariate analysis	Description	Equation	References
Magalef's species richness index (D)	total number of species (S) at a sampling site; N represents the total number of individuals	$D = \frac{S-1}{\ln(N)}$	(Margalef 1958)
Shannon–Wiener diversity index (H')	species diversity by considering the amount of species (i) relative to the total number of individuals (N)	$H' = -\sum p_i \times \ln(p_i)$ $p_i = \frac{n_i}{N}$	(Shannon & Weaver 2015)
Pielou's evenness index (J)	describes the biodiversity of a sample by the evenness of the species number (S)	$J = \frac{H'}{\ln(S)}$	(Pielou 1966)

Data exploration to test for assumptions for all regression analyses were conducted after Zuur et al. (2010). Regression analysis and a generalized additive model (GAM) was used to evaluate the relationship between $\partial^{13}C$ and $\partial^{15}N$ stable isotope values, respectively and mantle length, sex, maturity stage, C/N ratio and location for both gladii and muscle tissue. To fit the gladii increments to the ontogenetic stages, the gladius length was recalculated to the original mantle length. Therefore, the percentage ratio of the gladius length to the mantle length of each of the six individuals was calculated and the mean percentage ratio of the six individuals was used to recalculate the mantle length for each specific gladius increment and was assigned to the following five ontogenetic stages: Postlarvae (1 – 2.5 cm), early juveniles (3 – 9 cm), late juveniles (9 – 15 cm), adult middle sized (15 – 35 cm) and adult large sized (35 – 65 cm). An analysis of variance (ANOVA) was used to test whether the isotope values of the ontogenetic stages of all gladii were significantly different. All statistics were performed via the freeware RStudio (Version 3.3.2).

3 Results

3.1 Sex differences in *Sthenoteuthis pteropus*

During the three research cruises, 129 specimens were captured, comprising of 97 females (75%) and 32 males (25%). Females had a mean mantle length of 26.3 ± 6.1 cm and a mean weight of 752 ± 697 g, compared to a mean mantle length of 19.6 ± 2.1 cm and mean weight of 239 ± 88g in males. Most females were immature (77%) and ranged between 20 – 30 cm mantle length, followed by 13% mature and 6% maturing females (appendix Fig. 19 left). Male squid were mainly mature (75%), followed by 16% maturing and 9% immature individuals and mainly ranged between 18 – 21 cm mantle length (appendix Fig. 19 right).
The sample size of *S. pteropus* were 22, 50 and 57 for cruise M119, M116 and MSM49, respectively (appendix table 6).

3.2 General diet analysis

Stomach contents showed three main groups of prey: fishes, cephalopods and crustaceans (Fig. 6). A total of 346 otoliths and 27 beaks were found (Table 3). Over 80% of cephalopod and crustacean occurrences were single and not more than three individuals per stomach occurred. A third of fish occurrences were single or twice (in total 50%) and more than five individuals per stomach were rare. A stomach contained in average 3 species ± 1.9 and 9 species were the maximum found in one stomach.

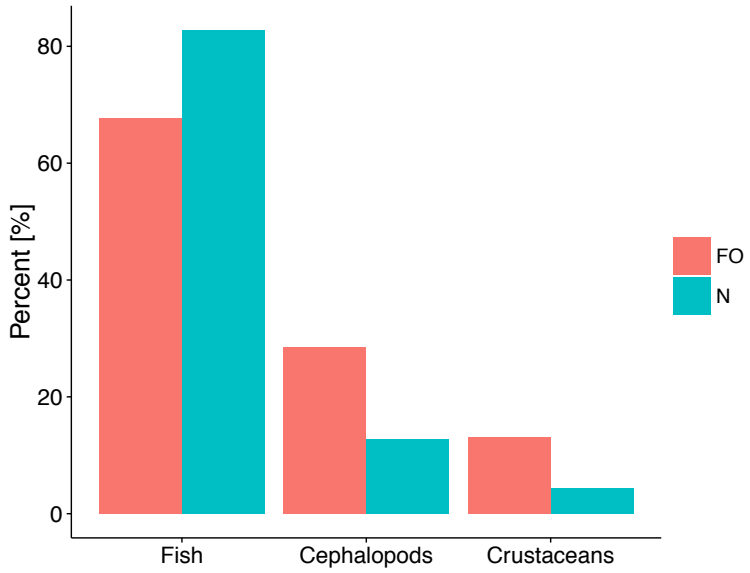

Figure 6. Frequency of occurrence (FO) and number (N) in percent of the three main prey items fish, cephalopods and crustaceans of *Sthenoteuthis pteropus* (n=129) caught in the eastern tropical Atlantic in 2015

About 68% of the observed stomachs contained fish, accounting for 83% by number (N). The most abundant family present in occurrence and number were Myctophidae. 30 different myctophid species were identified in the stomachs mainly represented by *Myctophum asperum* and *Myctophum nitidulum* (Fig. 7B).

15.8% of all fish otoliths could not be identified with an occurrence (FO) of 31.5%. Fishes of the genus *Vinciguerria* (Family: Phosichthyidae) accounted for 6.9 FO% and 10.1 N% although they were present in only nine stomachs, but in a quite high number (n=41). Other fish families found were Bathylagidae, Exocoetidae, Bregmacerotidae, Paralepididae, Stomiidae and Nomeidae (Fig. 7A). The maximum number of fish in a single stomach was 22. Especially *Vinciguerria attenuata* and *Vinciguerria nimbaria* were found in high numbers in one stomach of a female squid with 23.5 cm mantle length (n=22).

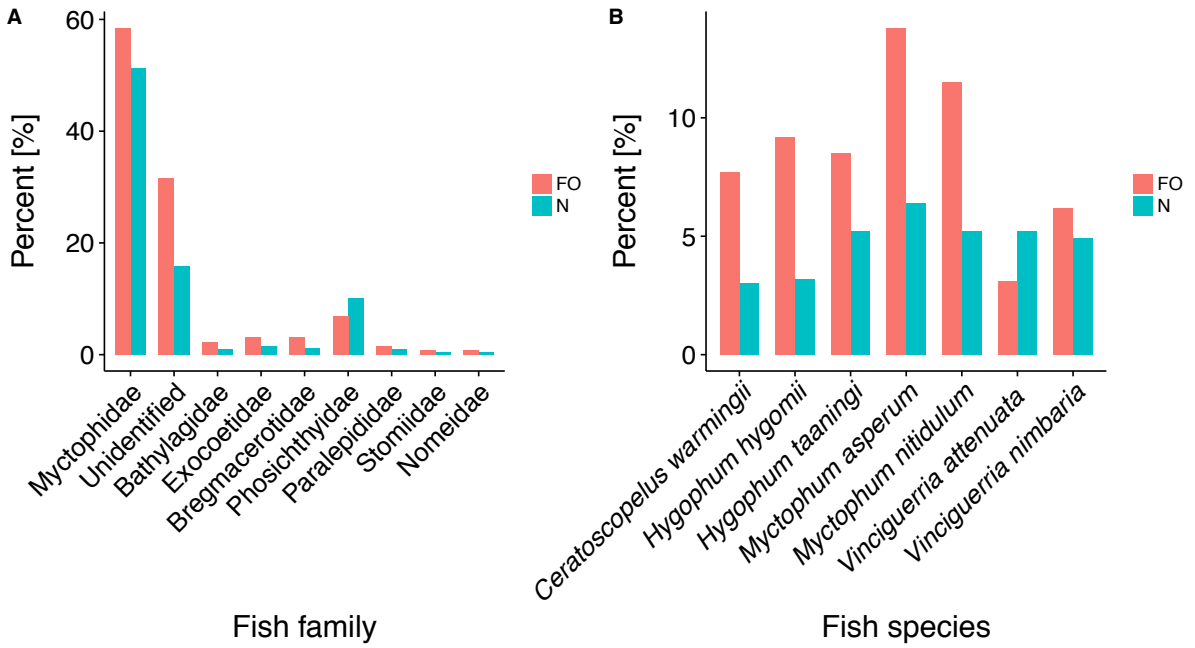

Figure 7. Frequency of occurrence (FO) and number (N) in percent of the stomach contents of *Sthenoteuthis pteropus* (n = 129) caught in the eastern tropical Atlantic in 2015; A: Fish families found in the stomachs; B: Most abundant fish species found in the stomach

Cephalopods were the second most important prey group of *Sthenoteuthis pteropus* (Fig. 8C) occurring in 29% of all stomachs and accounted for 13% of all prey by number. Since the beaks were mainly very small and eroded by digestion, they were identified to family level and not to species level to avoid bias. In contrast to the fish otoliths, the squid beaks consumed by *Sthenoteuthis pteropus* were not dominated by a single taxonomic group. Beaks that could not be identified accounted for the largest grouping (19%FO, 8%N). Approximately 2% of the lower beaks were identified as Enoploteuthidae (6% FO). Two beaks of cirrate octopods were found. The remaining 15% of beaks present in the stomachs belonged to the families Bolitinidae, Ommastrephidae, Pyroteuthidae, Mastigoteuthidae, Histioteuthidae and Onychoteuthidae with abundance and occurrence below 2%.

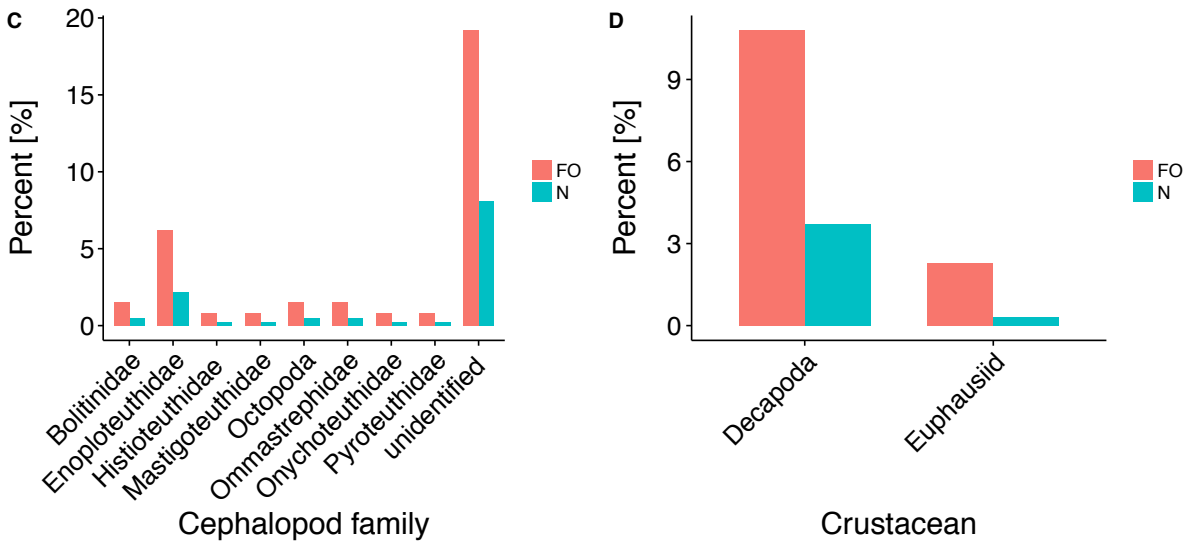

Figure 8. Stomach contents of *Sthenoteuthis pteropus* (n=129) caught in the eastern tropical Atlantic in 2015; C: Cephalopod families found in the stomachs; D: Crustaceans found in the stomach; FO = Frequency of occurrence (%); N = Number (%)

13% of all stomachs contained crustaceans, mainly decapods (FO% = 11%) and euphausiids (2% FO) (Fig. 8D). Decapods could not be identified to species level, because only small pieces of exoskeletons were found.

Small crustaceans such as copepods, isopods and amphipods as well as pelagic sea snails (Pteropoda) were excluded from the analysis, assuming them to be secondary or transitory prey.

Table 3. Summary of prey composition found in the stomach contents of *Sthenoteuthis pteropus* from the eastern tropical Atlantic in 2015 by frequency of occurrence (FO) and number (N).

Prey	FO	FO%	N	N%
Pisces	88	67.7	336	82.8
Myctophidae	76	58.5	208	51.2
Bolinichthys sp.	5	3.8	6	1.5
Ceratoscopelus warmingii	10	7.7	12	3.0
Diogenichthys atlanticus	1	0.8	1	0.2
Diaphus sp.	1	0.8	1	0.2
Diaphus dumerilii	5	3.8	12	3.0
Diaphus fragilis	2	1.5	2	0.5
Diaphus lucidus	1	0.8	1	0.2

Diaphus vanhoeffeni	9	6.9	9	2.2
Gonichthys sp.	4	3.1	4	1.0
Hygophum sp.	7	5.4	9	2.2
Hygophum hygomii	12	9.2	13	3.2
Hygophum macrochir	7	5.4	9	2.2
Hygophum taaningi	11	8.5	21	5.2
Hygophum proximum	3	2.3	4	1.0
Hygophum reinhardtii	3	2.3	9	2.2
Lampanyctus sp.	3	2.3	4	1.0
Lampanyctus intricarius	1	0.8	1	0.2
Lampanyctus festivus	1	0.8	1	0.2
Lampanyctus nobilis	2	1.5	2	0.5
Lepidophanes gaussi?	1	0.8	1	0.2
Lepidophanes guentheri	1	0.8	1	0.2
Myctophum sp.	10	7.7	16	3.9
Myctophum asperum	18	13.8	26	6.4
Myctophum nitidulum	15	11.5	21	5.2
Myctophum obtusirostre	1	0.8	1	0.2
Myctophum selenops	4	3.1	4	1.0
Myctophum spinosum	4	3.1	4	1.0
Notoscopelus caudispinosus	1	0.8	3	0.7
Symbolophorus sp.	4	3.1	5	1.2
Symbolophorus rufinus	4	3.1	5	1.2
Unidentified Myctophidae	18	13.8	22	5.4
Other Pisces				
Bathylagidae	3	2.3	4	1.0
Exocoetidae	4	3.1	6	1.5
Exocoetus sp.	3	2.3	3	0.7
Exocoetus obtusirostris	2	1.5	3	0.7
Bregmacerotidae	4	3.1	5	1.2
Bregmacerotidae sp.	1	0.8	1	0.2
Merluccius sp.	3	2.3	4	1.0
Phosichthyidae	9	6.9	41	10.1
Vinciguerria attenuata	4	3.1	21	5.2
Vinciguerria nimbaria	8	6.2	20	4.9

Paralepididae	2	1.5	4	1.0
Paralepididae sp.	1	0.8	1	0.2
Lestidiops sphyrenoides	2	1.5	2	0.5
Lestrolepis intermedia	1	0.8	1	0.2
Stomiidae	1	0.8	2	0.5
Chauliodus sloani	1	0.8	2	0.5
Nomeidae	1	0.8	2	0.5
Cubiceps pauciradiatus	1	0.8	2	0.5
Unidentified Otoliths	41	31.5	64	15.8
Cephalopoda	37	28.5	52	12.8
Bolitinidae	2	1.5	2	0.5
Ommastrephidae	2	1.5	2	0.5
Enoploteuthidae	8	6.2	9	2.2
Pyroteuthidae	1	0.8	1	0.2
Mastigoteuthidae	1	0.8	1	0.2
octopoda cirrata	2	1.5	2	0.5
Histioteuthidae	1	0.8	1	0.2
Onychoteuthidae	1	0.8	1	0.2
Unidentified Beak (destroyed or upper beak)	25	19.2	33	8.1
Crustacea	17	13.1	18	4.4
Decapoda	14	10.8	15	3.7
Euphausiid	3	2.3	3	0.7
Total	**130**		**428**	

Secondary Prey (Crustacea)	**28**	**214**
Copepoda	20	189
Amphipoda	7	9
Cladocera	3	4
Isopoda	2	2
Chaetognatha	1	3
Pteropoda	2	6
Algae	1	1
Total	**28**	**214**

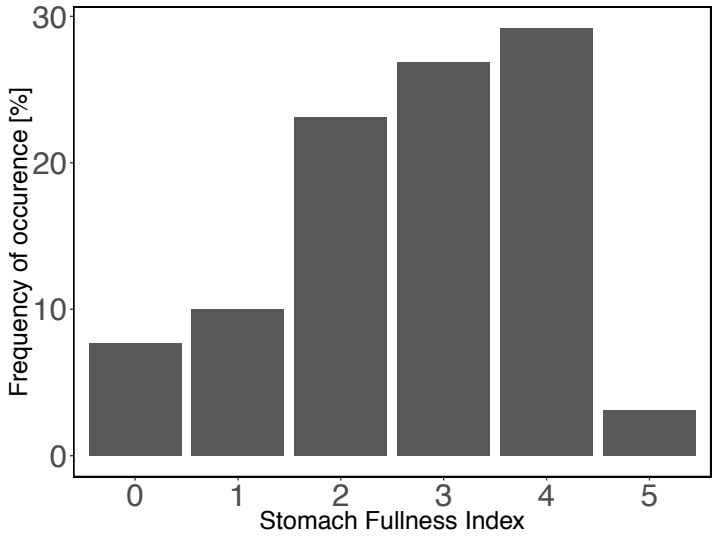

Figure 9: Frequency of occurrence in percent for the range of the stomach fullness indices of *Sthenoteuthis pteropus* in the eastern tropical Atlantic in 2015; 0 = empty, 1 = little traces of food, 2 = less than half full, 3 = more than half full, 4 = full, 5 = very full and exclusively crammed with the stomachs wall extending

Most stomachs were full accounting for 29%, followed by 27% stomachs that were more than half full and 23% that were less than half full (Fig. 9). Little traces of food were observed in 10% of all squid and 8% were empty. Just 3% of all stomachs were very full and exclusively crammed with the stomachs wall extending. No significant relationship was found between stomach fullness index and maturity stage, mantle length, sex or squid weight.

3.3 Differences in prey composition between three research cruises

During both cruises, M116 and MSM49, a total of 36 different species were observed in the stomachs of *Sthenoteuthis pteropus* whereas during cruise M119 only 27 species occurred (Table 4). This was probably due to the smaller sample size of captured squid during the cruise M119 (n_{M119} = 22, in comparison n_{M116} = 50 and n_{MSM49} = 57).

Table 4. Comparison of the dominance and diversity indices of the prey composition of *Sthenoteuthis pteropus* (n=129) from the research cruises MSM49, M116 and M119 in 2015

	MSM49	M116	M119
No. of species	36	36	27
Magalef's species richness (D)	9.269	9.126	7.430
Pielou's evenness (J)	0.993	0.992	0.992
Shannon's diversity [H']	3.586	3.582	3.271

Magalef's species richness (D) showed higher values for the cruises MSM49 and M116 as well as Shannon's diversity index, probably also due to the smaller sampling size during M119. Pielou's evenness index (J) was similar for all cruises. No significant differences in species composition between the three cruises were present. However, the two cruises with the largest geographical distance in between, cruises MSM49 and M119, showed the highest dissimilarity of 48.2%. Mainly *Vinciguerria attenuata, Myctophum spinosum* and crustaceans led to the largest differences (appendix table 9). These species were present in the squid stomachs of the cruise MSM49, and not in M119. In comparison, the dissimilarity between MSM49 and M116 was only 35.2% mainly due to the species *Hygophum reinhardti* and *Hygophum proximum* that were present in M116 and not in MSM49 (appendix table 8). Cruises M116 and M119 exhibited an average dissimilarity of 45.4% mainly due to *Myctophum spec*., *Hygophum sp.*, *Hygophum reinhardti* and *Hygophum macrochir* present in M116 (appendix table 10), comparable to the differences between MSM49 and M119. This is in accordance with the dominance and diversity indices presented above (Table 5) showing lower species diversity in prey composition in M119 compared to MSM49 and M116. In general, the prey composition of all cruises was dominated by myctophids (Table 5). Cruise MSM49 revealed the highest fish and cephalopod diversity. Fish species of the families Nomeidae and cephalopod beaks of the families Mastigoteuthidae, Histioteuthidae, Onychoteuthidae and of unidentified cirrate octopods were only found in squid stomachs of this cruise. In M119 crustaceans did not occur.

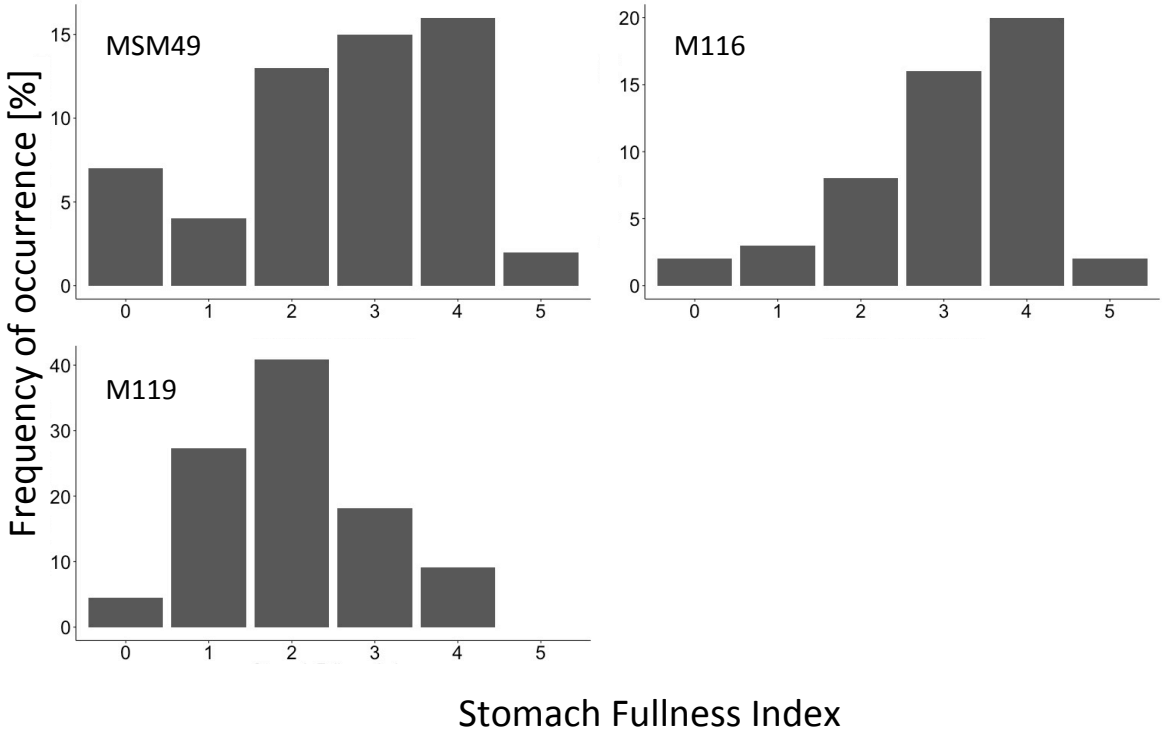

Figure 10. Frequency of occurrence in percent of the stomach fullness indices of *Sthenoteuthis pteropus* of the cruises MSM49 (A), M116 (B) and M119 (C); 0 = empty, 1 = little traces of food, 2 = less than half full, 3 = more than half full, 4 = full, 5 = very full and exclusively crammed with the stomachs wall extending

The analyzed squid were grouped according to the three research cruises MSM49, M119 and M116. All expeditions showed the same patterns in stomach fullness index distribution (Fig. 10). Only slight differences were visible. Most of the stomachs of MSM49 and M116 were more than half full or full whereas most stomachs of M119 were less than full. MSM49 had the highest frequency of occurrence of empty stomachs.

Table 5. Frequency of occurrence and number of major prey groups of *Sthenoteuthis pteropus* separated by cruises

	M119				M116				MSM49			
	FO	**FO%**	**N**	**N%**	**FO**	**FO%**	**N**	**N%**	**FO**	**FO%**	**N**	**N%**
Pisces	**14**	**63.6**	**51**	**76.1**	**43**	**86.0**	**157**	**83.5**	**31**	**54.4**	**107**	**82.3**
Myctophidae	14	63.6	42	56.0	38	76.0	126	60.9	25	43.9	63	43.2
Bathylagidae					1	2.0	1	0.5	2	3.5	3	2.1
Exocoetidae	2	9.1	2	2.7	1	2.0	1	0.5	1	1.8	3	2.1
Bregmacerotidae					2	4.0	2	1.0	2	3.5	3	2.1
Phosichthyidae	1	4.5	1	1.3	4	8.0	15	7.2	4	7.0	25	17.1
Paralepidae					1	2.0	1	0.5	1	1.8	3	2.1
Stomiidae					1	2.0	2	1.0				
Nomeidae									1	1.8	2	1.4
Unknown otoliths	5	22.7	6	8.0	6	12,0	9	4.3	4	7.0	5	3.4
Cephalopoda	**9**	**40.9**	**16**	**21.3**	**17**	**34.0**	**22**	**10.6**	**11**	**19.3**	**14**	**9.6**
Bolitinidae					1	2.0	1	0.5	1	1.8	1	0.7
Ommastrephidae	1	4.5	1	1.3	1	2.0	1	0.5				
Enoploteuthidae	5	22.7	5	6.7	1	2.0	2	1.0	2	3.5	2	1.4
Pyroteuthidae	1	4.5	1	1.3								
Mastigoteuthidae									1	1.8	1	0.7
octopoda cirrata									2	3.5	2	1.4
Histioteuthidae									1	1.8	1	0.7
Onychoteuthidae									1	1.8	1	0.7
unidentified Beak	6	27.3	9	12.0	15	30.0	18	8.7	4	7.0	6	4.1
Crustacea	**0**	**0.0**	**0**	**0.0**	**8**	**16.0**	**9**	**4.3**	**8**	**14.0**	**9**	**6.2**

3.4 Primary or secondary prey?

Smaller crustaceans (e.g copepods, amphipods, isopods and ostracods) are probably mainly introduced to the squid's stomachs by its prey (Markaida & Sosa-Nishizaki 2003). These taxa were considered secondary or transitory prey and were not included in the prey composition analysis. However, high numbers of intact copepods were found in twenty stomachs (n = 189, Table 3) without any evidence of recently digested prey. They occurred almost exclusively in squid caught during May and June (M116). The mantle length of squid containing more than one intact copepod specimen ranged from 15 to 25 cm. The maximum number of copepods found was 82 swallowed by a female squid of 25 cm mantle length.

3.5 General diet during the ontogeny of *Sthenoteuthis pteropus*

The variability of prey occurrence divided into four squid size classes of 10 cm length intervals is shown in figure 11. Fishes in general had the main occurrences in all squid sizes, mainly made up by myctophids (40 – 64%).

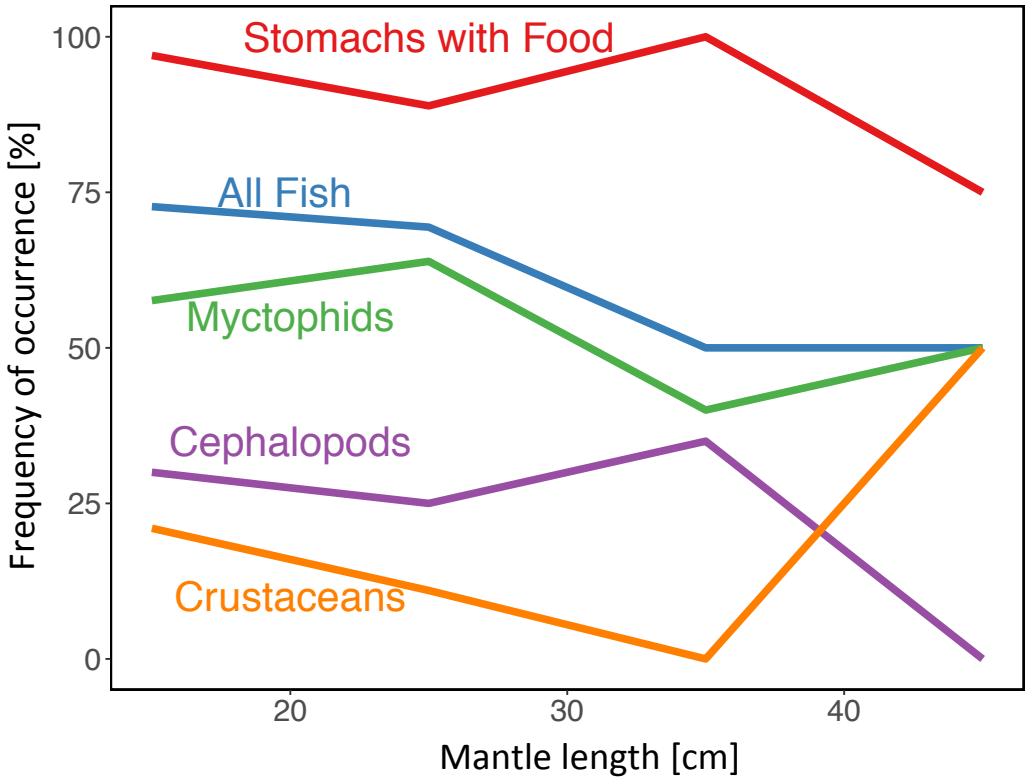

Figure 11. Frequency of occurrence of the prey groups of 129 specimens of *Sthenoteuthis pteropus* for 10 cm size intervals; sample size per size interval: 11 – 20 cm =33; 21 – 30 cm = 72; 31 – 40 cm = 20; 41 – 50 cm = 4

Phosichthyidae had small occurrences in squid between 15 to 30 cm (6 – 15%) and were totally absent in squid larger than 30 cm (Fig. 12). Squid larger than 35 cm did not feed on other fishes than myctophids except for one individual larger than 45 cm that fed on flying fish (Exocoetidae). These findings have to be used with caution, because the sample size for the largest size group (41 – 50 cm ML) is small and the feeding behavior is based on only four individuals.

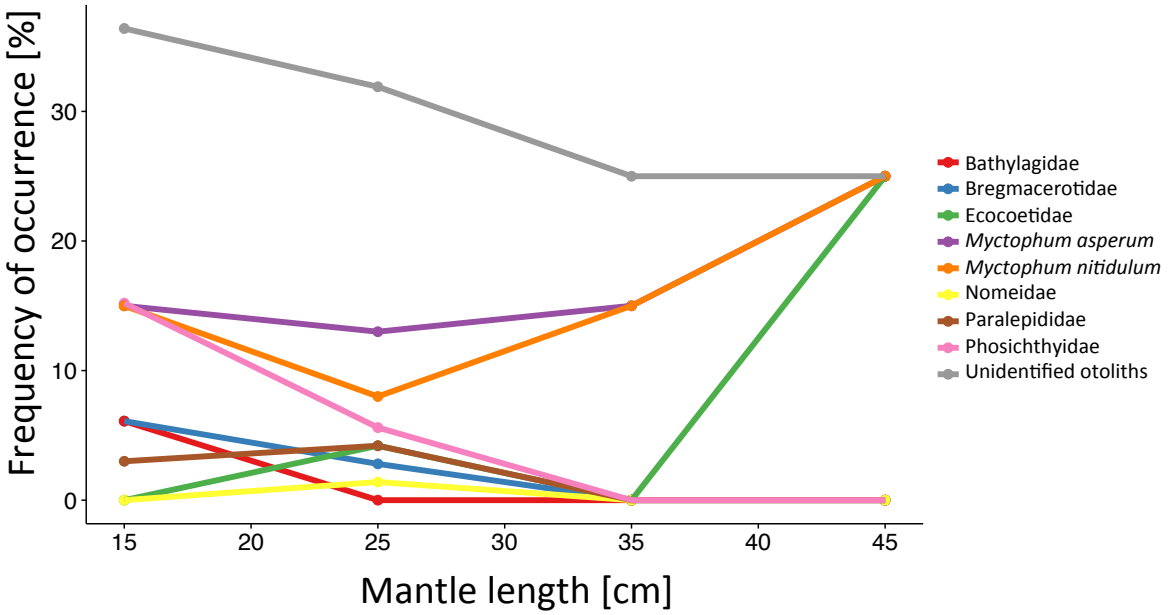

Figure 12. Frequency of occurrence of fish species in the stomachs of *Sthenoteuthis pteropus* (n=129) caught in the eastern tropical Atlantic in 2015 against its mantle length grouped into 10 cm size intervals; sample size per size interval: 11 – 20 cm =33; 21 – 30 cm = 72; 31 – 40 cm = 20; 41 – 50 cm = 4

Cephalopod prey was moderate (24– 35%) in *Sthenoteuthis pteropus* smaller than 40 cm and lacking in squid larger than 40 cm (Fig. 11). In squid larger than 40 cm no cephalopods were present. Again the small sample size has to be mentioned. Stomachs of squid in all size intervals contained food (75 – 100%). Crustaceans had the lowest frequency of occurrence compared with the other main prey groups ranging from 11 – 21 % in squid between 15 – 30 cm mantle length and 50% in squid larger than 40 cm. Squid between 30 – 40 cm did not feed on crustaceans.

3.6 Intraspecific structure

All collected squid have been organized into a northern and equatorial group based on the location of their catch and the classification of Zuyev & Nikolsky (1993). Squids caught at latitudes higher than 12°N have been assigned to the northern form and squid caught at latitudes lower than 12°N have been assigned to the equatorial form. The mantle length between these two groups is significantly different ($p<0.01$; $F=9.23$). Squid in the northern group (n = 44) were on average 3 cm larger (mean = 26.7 ± 7.3 cm ML) than squid caught in the equatorial region (23.4 ± 5 cm ML; n = 85). The maximum mantle length found in the northern region was 47.5 cm, and 39 cm in the southern region. The mantle length at maturity in female squid also differed. Female squid from the northern region reached a mantle length of 45 ± 2.1 cm when they were mature. However, these findings are based on only two individuals. The mantle length of female mature squid in the equatorial region was 27 ± 4 cm on average (n=8). Mature male squid showed no differences in mantle length with 19.9 ± 0.8 cm and 20.1 ± 2 cm in the northern and equatorial group, respectively.

3.7 Stable isotope analysis

3.7.1 Stable isotope analysis of muscle tissue

The C/N ratio (ratio of carbon to nitrogen in the tissue) was consistent among individuals in both gladii (range: 4.0 – 4.8) (Table 6) and muscle samples (3.4 – 3.8) (appendix table 11) which indicates a similar biochemical composition of tissues and allows inter- and intra-individual comparisons. $\partial^{15}N$ and $\partial^{13}C$ gladii isotope values covered a large range from 5.3 – 9.2‰ (range: 3.9‰) and -18.5 - - 15.4 ‰ (range: 3.1‰), respectively (Table 6).

Muscle isotope values showed a smaller range for $\partial^{13}C$ ranging from -17.3 – -14.8‰ (range: 2.5 ‰) (Fig. 13B) and $\partial^{15}N$ values were higher in muscle than in gladii, due to the chitin content in gladii, which lowers $\partial^{15}N$, and ranged from 9.7 – 13.3‰ (3.6‰) (Fig. 13A). This range of 3.6‰ in $\partial^{15}N$ is equivalent to an increase by one trophic level (Minagawa & Wada 1984). A significant effect of size (mantle length) was found on muscle $\partial^{15}N$ isotope values (GAM, edf = 3.5, $F_{1,54} = 26.4$, $p<0.01$, deviance explained = 67.6%, $r^2 = 0.65$). $\partial^{15}N$ values increased almost exponentially with increasing mantle length in squid from 18 to 40 cm. In squid larger than 40 cm $\partial^{15}N$ seemed to stay constant or even decrease, but this has to be interpreted with caution, since only three individuals have been measured.

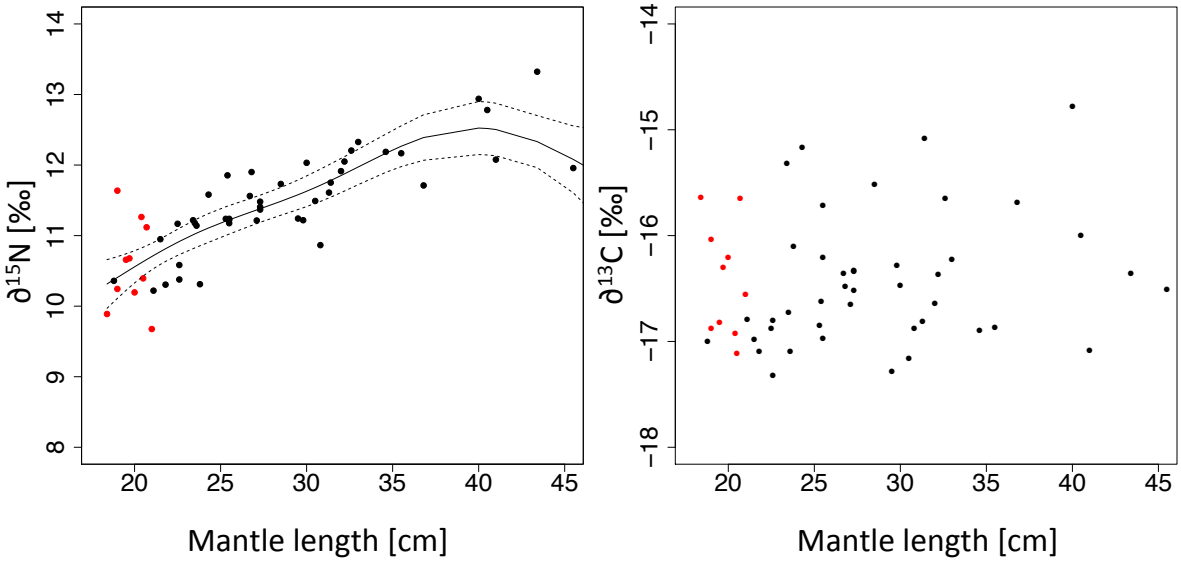

Figure 13. Isotopic values of muscle tissue (n = 54) of *Sthenoteuthis pteropus* caught in the eastern tropical Atlantic in 2015; left = ∂^{15}N in ‰ of muscle tissue; GAM, edf = 3.5, $F_{1,54}$ = 26.4, p<0.01, deviance explained = 67.6%, r^2 = 0.65; right = ∂^{13}C in ‰ of muscle tissue; red dots = males, black dots = females

No relationship was found between ∂^{15}N muscle isotope values and sex, maturity stage, C/N ratio and location. For ∂^{13}C no relationship was found between the covariates mentioned above (y = 0.01 - 16.1x , p = 0.39, r^2 = 0.01, F_{1-52}= 0.74). The ∂^{15}N muscle isotope values of females and males were significantly different, with females having a higher average ∂^{15}N by 1 ‰ (11.5 ± 0.7 ‰, n = 43) than males (10.6 ± 0.6‰, n = 10) (Welch two sample t-test: p < 0.01, t = 4.08) (Fig. 14 right). However, the sample size for males is low (n=10). This significant difference between females and males is not present in ∂^{13}C (Fig. 14 right). The largest individual (Individual C; 47.5 cm) showed a lower ∂^{15}N value (11.3 ‰) than all other individuals larger than 40 cm (> 12.1 ‰) (Table 6).

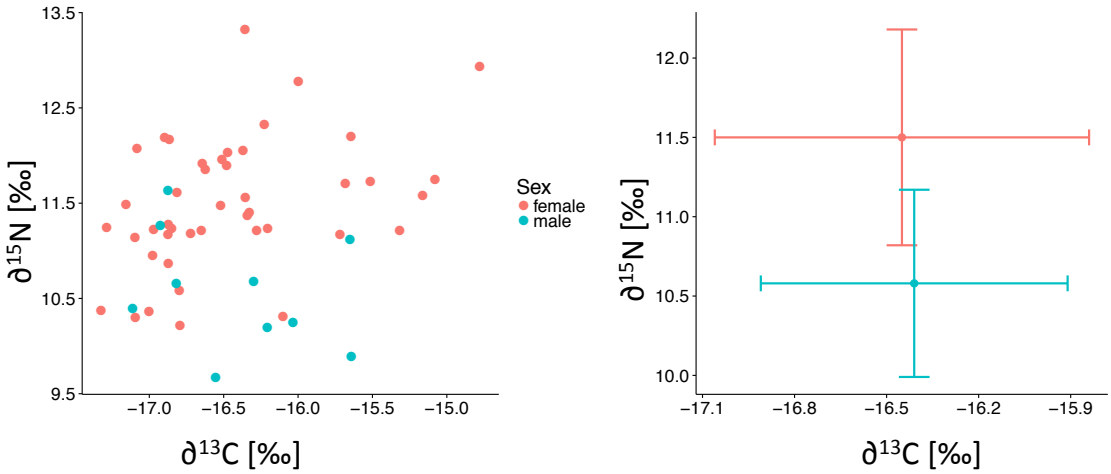

Figure 14. Stable isotopes of muscle tissue (n = 54) of *Sthenoteuthis pteropus* caught in the eastern tropical Atlantic in 2015; left = $\partial^{15}N$ and $\partial^{13}C$ of muscle tissue; right = mean and standard deviation of $\partial^{15}N$ and $\partial^{13}C$ of muscle tissue of females (n = 44) and males (n = 10)

3.7.2 Stable isotope analysis of squid gladii

Gladii isotope data and mantle length (Table 6, Fig. 15) showed a weak relationship for $\partial^{15}N$ ($y = 7.13 + 0.02x$, $p < 0.01$, $r^2 = 0.06$, $F_{1, 133} = 8.52$) and also a weak relationship for $\partial^{13}C$ ($y = -16.88 + 0.02x$, $p < 0.01$, $r^2 = 0.13$, $F_{1, 133} = 20.17$). The overall fit of the regression model was very weak and mantle length only accounted for 6% and 13% of the variation in $\partial^{15}N$ and $\partial^{13}C$, respectively.

The life cycle of *Sthenoteuthis pteropus* can be divided into five ontogenetic stages (Zuyev et al. 2002) dependent on its mantle length and food spectrum (see Introduction, Table 1, ontogenetic stage “Paralarvae” excluded). In this study, the gladius length of each section measured has been recalculated to its original mantle length to compare the isotope values between the individual ontogenetic stages (Figure 15). Individual C, the largest squid analyzed, showed a continuous increase in $\partial^{15}N$ of 3‰ (5.2‰ $\partial^{15}N$ at 22.1 cm ML to 8.5‰ $\partial^{15}N$ at 47.5 cm ML) as it reached 20 cm mantle length accompanied by an increase of $\partial^{13}C$.

Table 6. Size, location of capture, isotope values and C/N mass ratios of the five large *Sthenoteuthis pteropus* and the small individual caught during the cruise MSM49 in 2015 in the eastern tropical Atlantic (S = Senghor Seamount, Ref = Reference Station).

			Mantle			Gladius								
			$\partial^{15}N$	$\partial^{13}C$	C/N	$\partial^{15}N$			$\partial^{13}C$			C/N		
Individual	**Size [cm]**	**Stations**				**mean**	**max**	**min**	**mean**	**max**	**min**	**mean**	**max**	**min**
A	45.5	S. Ref	11.9	-16.5	3.7	7.0	8.0	6.2	-16.9	-15.7	-17.8	4.5	4.7	4.3
B	40.5	S. Slope SE	12.8	-16.0	3.4	7.6	8.0	7.3	-16.4	-16.1	-17.3	4.5	4.7	4.4
C	47.5	S. Slope SE	11.3	-16.9	3.7	6.5	8.6	5.3	-16.7	-16.0	-17.1	4.5	4.6	4.4
D	41.0	S. Summit	12.1	-17.1	3.8	7.8	8.3	7.4	-17.7	-16.0	-18.5	4.4	4.8	4.3
E	43.4	Eddy Core	13.3	-16.4	3.6	8.3	9.2	7.5	-16.1	-15.4	-17.5	4.4	4.6	4.0
F	21.0	S. Ref	9.7	-16.6	3.5	6.5	7.6	5.9	-16.7	16.4	-17.2	4.6	4.6	4.5

Individual A showed fluctuating isotopic values with decreasing and increasing $\partial^{15}N$ and $\partial^{13}C$. Opposite to Individual A, Individual D showed almost no fluctuation in $\partial^{15}N$, but a steady increase of $\partial^{13}C$ of -18 ‰ to -17‰. The $\partial^{15}N$ and $\partial^{13}C$ values of all individuals revealed that the trophic position and foraging areas varied at short time intervals over their entire life span (Fig. 15). However, all large individuals also showed the same pattern as $\partial^{15}N$ and $\partial^{13}C$ slowly increased from a mantle length larger than of 30 cm. This mantle length can be assigned to the ontogenetic stages 5 and 6, from middle-sized adults to large-sized adults, in which the second dietary shift is supposed to happen from micronektonic fishes to nektonic fishes and squids.

The isotopic values of the male individual (Individual F) reside between the females and also show high variability. No significant relationship was present between the stable isotope values in gladii and muscle tissue and the location of capture.

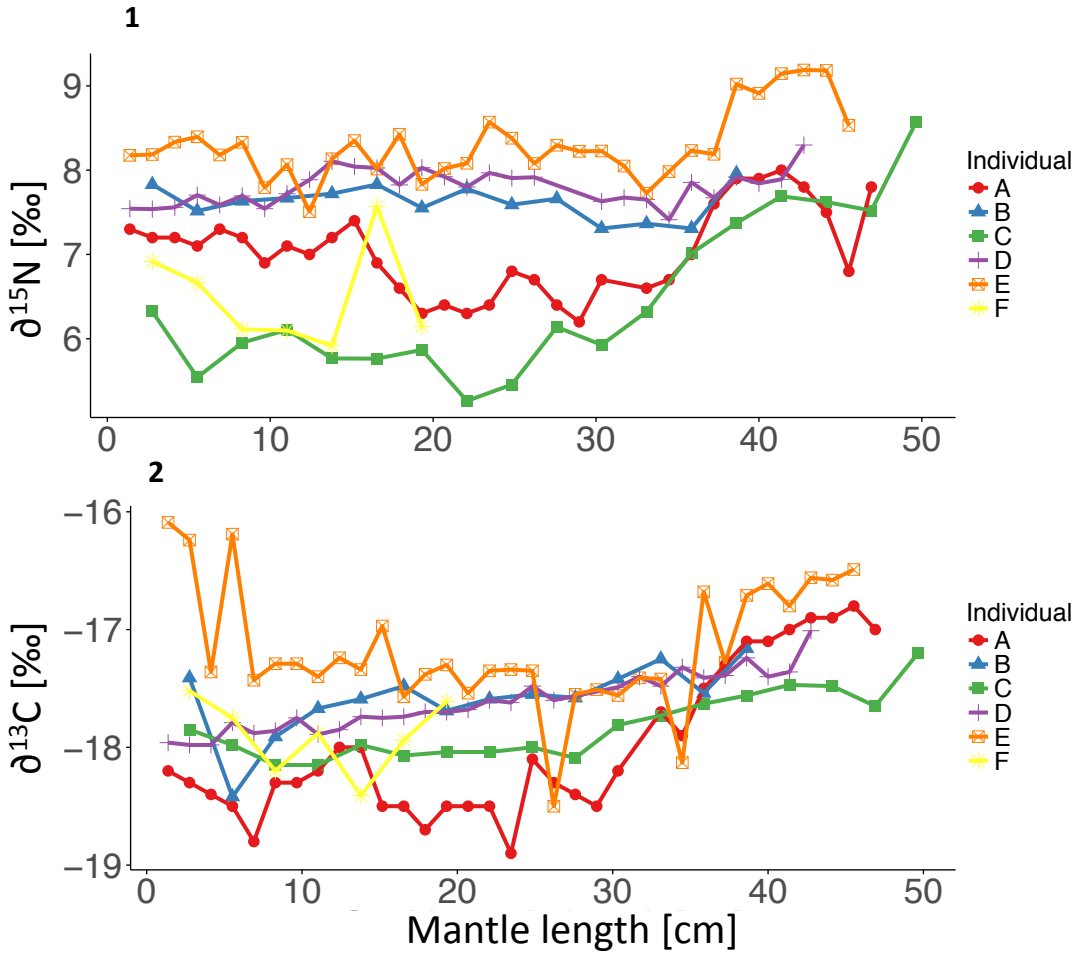

Figure 15: ∂^{15}N (1) and ∂^{13}C (2) stable isotope values along the mantle length of the five large female *Sthenoteuthis pteropus* (A – E) and the small male individual (F) caught in the eastern tropical Atlantic in 2015

The ∂^{15}N isotopic values of the first four ontogenetic stages (postlarvae – adult, middle sized squid) did not differ significantly (Fig. 16B). The middle sized ($p = 0.02$) and late juveniles ($p = 0.02$) were weakly significantly different to the large sized stage (ANOVA: $F_{1,4} = 4.243$, $p < 0.01$). However, the different stages overlapped to a high degree and a real separation between different trophic positions was not visible. The isotopic values in ∂^{13}C of the different ontogenetic stages varied more than in ∂^{15}N (Fig. 16A). Early juveniles ($p < 0.001$), late juveniles ($p < 0.001$) and middle sized squid ($p < 0.001$) showed significant smaller ∂^{13}C isotopic values than large sized squid (ANOVA: $F_{1,4} = 12.68$, $p < 0.001$).

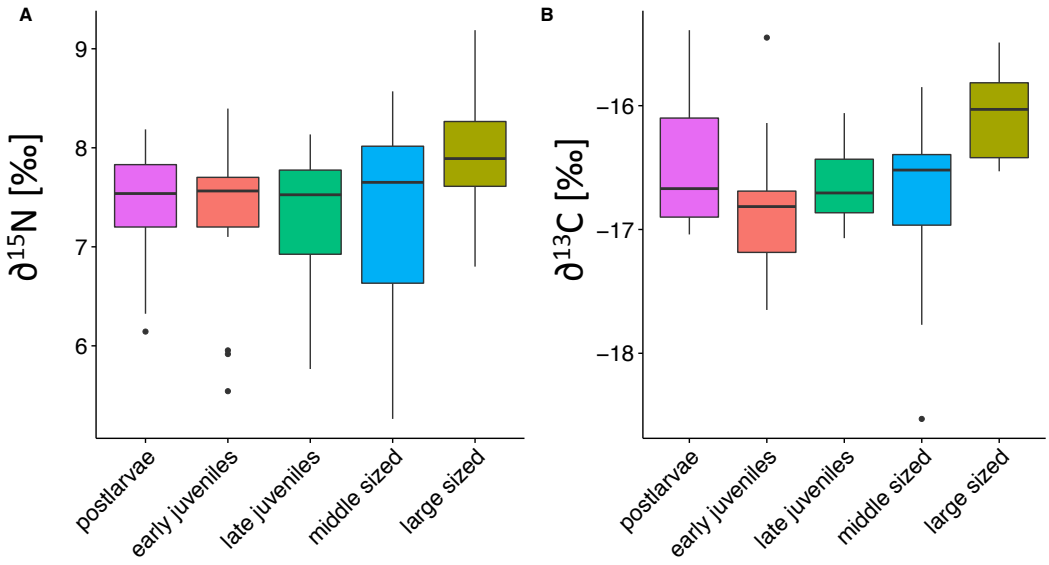

Figure 16. Gladii stable isotope values of the five large female *Sthenoteuthis pteropus* caught in the eastern tropical Atlantic in 2015 grouped into five ontogenetic stages. postlarvae = 1.0 – 2.5cm ML, early juveniles = 3 – 9 cm ML, late juveniles = 9 – 15 cm ML, middle sized = 15 – 35 cm ML, large sized = 35 – 65 cm ML

4 Discussion

This study on the diet and trophic ecology of *Sthenoteuthis pteropus* revealed three main findings. First, stomach content data showed that juvenile and adult *S. pteropus* mainly prey on myctophids, but that they also show an opportunistic and variable feeding behavior depending on which prey is available. In the diet, no ontogenetic shift in prey composition was present. Second, the muscle tissue stable isotope analysis exhibits an overall increase in trophic position during ontogeny by approximately one trophic level between juvenile and adult life stages. The $\partial^{13}C$ isotopic values did not show any trend with increasing mantle length, stressing the individual migratory behavior of this species. Third, by reconstructing the feeding chronology along the gladii of individual squid we did not observe a continuous increase in trophic position during their whole life cycle, but a continuous increase in $\partial^{15}N$ when squid exceeded 20 – 30 cm mantle length as seen in the muscle tissue. Furthermore, substantial variations in their trophic position and foraging area were observed. The results suggest that stomach content and muscle tissue stable isotope analysis alone are not sufficient to identify fine-scale spatiotemporal variations in the trophic ecology of squid. They should be complemented by stable isotope analysis of archival hard body parts, such as the gladius, that grow with the animal and display the feeding and foraging chronology of an individual squid.

4.1 Sex differences in *Sthenoteuthis pteropus*

Males and females of *Sthenoteuthis pteropus* have significantly different mantle lengths. Males can reach a maximum mantle length of 35 cm, whereas females grow up to 65 cm (Zuyev et al. 2002). This sexual dimorphism is confirmed in the present study and in other ommastrephid squids such as *Ommastrephes bartramii* and *Sthenoteuthis oulaniensis* (Arkhipkin & Bizikov 1991, Arkhipkin & Mikheev 1992). The average female caught in the eastern tropical Atlantic in 2015 during this study had a mantle length of 26.3 ± 6.1 cm and the average male was significantly smaller with 19.6 ±2.1 cm.

Additionally, females live for about one year and males are expected to have a lifespan that is about 1 – 2 months shorter (Zuyev et al. 2002). Males grow slower than females and become mature earlier. At an age of 90 – 120 days sexual dimorphism appears when males begin maturing and their growth rate decreases from 7-8% body weight d^{-1} to 2.4 – 3% body

weight d^{-1} at the age of 120 – 180 days. The growth rate of mature males at the age of 210 – 270 days is even lower (0.4 – 1.0% body weight d^{-1}). Females begin maturing at an age of 200 – 220 days (around 100 days later than males) and are probably completely mature at an age of 300 – 320 days. The growth rate of mature females is also higher than in males (1.8 – 2.5% body weight d^{-1}). The early maturation of males causes this decrease in growth rates and this leads to different sizes between males and females in the last phase of their life at an age of 270 – 350 days and to different trophic levels (Arkhipkin & Mikheev 1992), enabling them to avoid intersexual competition for food. However, growth rates are influenced by seasonal differences in environmental conditions, different foraging areas and different cohorts (Arkhipkin & Mikheev 1992).

4.2 General diet analysis

Research Question 1: What is the ecological role and general diet of Sthenoteuthis pteropus *in the food web of the eastern tropical Atlantic?*

The diet of *Sthenoteuthis pteropus* consisted mainly of myctophids, the most abundant fish group in all oceans. Myctophids also form the major prey of many other ommastrephid squids such as *Dosidicus gigas* (Markaida & Sosa-Nishizaki 2003, Markaida 2006), *Ommastrephes bartramii* (Nigmatullin et al. 2009), *Sthenoteuthis oualaniensis* (Parry 2006), *Nototodarus sloanii* (Pethybridge et al. 2012), *Todarodes sagittatus* (Piatkowski et al. 1998) and also of e.g. Onychoteuthidae such as *Moroteuthis ingens* (Jackson et al. 1998, Phillips et al. 2003).

30 different myctophid species were found in the stomachs of *S. pteropus*, especially *Myctophum asperum* and *Myctophum nitidulum. M. asperum* and *M. nitidulum* belong to the most dominant surface-migratory myctophid fishes in the tropical and sub-tropical Pacific (Watanabe et al. 2002), but few information is available about these species in the Atlantic. *M. asperum* can reach a maximal length of 8.5 cm and mainly preys on polychaetes, ostracods, appendicularians, copepods, euphausiids and amphipods (Watanabe et al. 2002, Sassa & Kawaguchi 2004). *M. nitidulum* covers the same size range with a maximum length of 8.3 cm and mainly preys on copepods and amphipods (Watanabe et al. 2002). *M. asperum* changes its feeding habits from diurnal, visual feeding to nocturnal feeding after metamorphosis (Sassa & Kawaguchi 2004). Adults of both species feed mainly in the

epipelagic zone at night within the upper 1 m layer. The vertical distribution pattern of *S. pteropus* therefore follows its main prey. Late juveniles and adult squid rise to the epipelagic layer at night to forage (0 – 150 m) and descend down to 800 - 1200 m in the morning (Zuyev et al. 2002). Paralarvae, postlarvae and early juveniles up to 9 cm mantle length do not perform extensive diurnal vertical migrations and stay in the surface homogenous surface layer between 50 – 75 m. The sister species *Sthenoteuthis oualaniensis* shows a surprising phenomenon in the Arabian Sea. Giant females actively feed on dense concentrations of mesopelagic fishes in depths of 200 – 350 m during the day probably because of the favorable feeding conditions (Zuyev et al. 2002). In the Mediterranean Sea and the Pacific Ocean tuna and dolphin fishes also feed on *Myctophum* species (Olson & Galván-Magaña 2002, Karakulak et al. 2009). Due to the high abundance in the Atlantic we can assume *Myctophum* species to be an important prey for higher trophic levels, but more research is needed to validate this assumption.

Nine of all myctophid species found in the stomachs of *S. pteropus* only occurred once in a single stomach, stressing their high versatility in choosing prey. This variance in prey species, also seen in other squid species (Hunsicker et al. 2010), could be explained by the migratory behavior of *S. pteropus*. However, many different myctophid species are able to coexist due to resource-partitioning of vertical distribution and diet and therefore face reduced trophic competition (Hopkins & Gartner 1992, Shreeve et al. 2009). Therefore the variance in prey species could also be due to an assembly of different myctophid species.

Myctophids in general are characterized by high biomasses (Gjøsaeter & Kawaguchi 1980) and occur in mesopelagic and bathypelagic waters of all oceans. They can perform extensive vertical diel migrations from the deep sea to the sea surface at night to forage (Gjøsaeter & Kawaguchi 1980, Catul et al. 2011), therefore occupying an important role in transferring energy from sea surface layers to the deep. Besides their high abundance and widespread distribution, myctophids show a high lipid content and form a higher energetic value as prey than several other fish species (Saito & Murata 1998, Lea et al. 2002). They support many pelagic predators of higher trophic levels including squaloid sharks, gadoids, merluciids such as hake, swordfish and some tuna species (Perrin et al. 1973, Ebert et al. 1991, Cherel et al. 1993, 1997, Phillips et al. 2001, Ménard et al. 2006, Cartes et al. 2009, Battaglia et al. 2013, Modica et al. 2015). Therefore, this study provides evidence that *S. pteropus* competes with large predatory fishes of higher trophic levels.

Rodhouse and Nigmatullin (1996) suggest that in the open ocean most predation pressure on fish stocks originates from epipelagic ommastrephid squids, and they conclude that the role of ommastrephid squids as predators and their transfer of energy and nutrients from the mesopelagic food web to higher trophic levels may be underestimated. Furthermore, *S. pteropus* has the potential to influence natural mortality and recruitment success in commercially valuable fish stocks due to its high abundance, short life cycle and opportunistic feeding behavior. The impact of a squid population on a commercial valuable fish stock is well illustrated in the case of *D. gigas.* This species migrates into the Gulf of California during summer months shifting its diet from myctophids, red crabs and conspecifics in winter to penaeid postlarvae and sardines in summer. Sardines account for 80% of their total diet at the end of summer which led to a decline in sardine fisheries in 1981 probably due to this increased predation pressure (Rodhouse & Nigmatullin 1996). Overexploitation of fish stocks may also be beneficial for squids as reported from the Pacific Ocean for *D. gigas* (Field et al. 2007, Zeidberg & Robison 2007). In the Atlantic Ocean the bluefin tuna (*Thunnus thynnus*) is heavily overfished (Hutchings 2000, Taylor et al. 2011, Fromentin et al. 2014). It also preys on myctophids (Battaglia et al. 2013) and the reduced competition for shared prey species between *S. pteropus* and *T. thynnus* caused by the overfishing of the bluefin tuna may lead to shifts in the population dynamics of *S. pteropus*. Its high plasticity, short life cycle and opportunistic feeding behavior will allow this squid to respond quickly to niches made accessible by the reduction of large predatory fishes.

S. pteropus and other squid species are important prey for marine top predators and themselves are important predators of lower trophic levels. Griffith et al. (2010) demonstrated that small numbers of species with very large biomasses and very high reproduction rates can lead to a combined bottom-up and top-down control manifested as a form of “wasp-waist” control within the pelagic ecosystem off eastern Australia that potentially causes unpredictable ecological responses if their biomasses are altered (Cury et al. 2000). This already may have happened in the eastern Pacific, where tuna and billfish populations declined due to fisheries (Myers & Worm 2003) and have been replaced by smaller individuals, smaller fish species and by squids, a consequence also seen in other fisheries (Caddy & Rodhouse 1998).

Besides fishes, *Sthenoteuthis pteropus* also preyed upon cephalopods and crustaceans. Cephalopods and crustaceans mostly occurred single in the stomachs of the squid, whereas fish remains mostly occurred in higher numbers, indicating the voracious feeding behavior of *S. pteropus* feeding on whole groups of fishes. However, fish otoliths in the stomachs could be accumulated during several meals leading to bias in frequency of occurrence and number (Markaida & Sosa-Nishizaki 2003).

Sthenoteuthis pteropus showed no relationship between stomach fullness index and maturity stage, mantle length, sex or weight. This indicates that *S. pteropus* continues feeding through its entire life regardless of the factors mentioned above. There is evidence, that squid of the genus *Sthenoteuthis* spawn multiple times and continue to feed, grow and mature additional oocytes between the different spawning events (Harman et al. 1989, Zuyev et al. 2002). This would explain the missing relationship between stomach fullness index and maturity stage, mantle length and weight.

4.2 Differences in prey composition between three research cruises

Research Question 2: Are there differences in prey composition between three research cruises?

The differences in abundance and species diversity between the three cruises taking place at different locations may be due to the varying sample size. Cruise M119 had the lowest sample size therefore less squid stomachs contributed to the analysis. Additionally, cruise M119 caught squid in a more southern equatorial region whereas MSM49 and M116 covered the northern equatorial region. Probably the different sampling locations also contributed to the differences in abundance and diversity of cephalopods. A relationship between abundance and species richness and seasons could not be observed since M119 took place during September/October and the other two cruises during December (MSM49) and May/June (M116). Due to the fact that climatic conditions and seasons do not change substantially in the equatorial region, we did not expect significant variation.

Cruise M119 showed the highest dissimilarity in prey composition compared to cruise M116 and MSM49. This is in accordance with the higher species richness and diversity seen in MSM49 and M116 compared to M119. However, it is not clear whether these differences

are a result of the different locations, seasons or sample sizes. Squid stomachs collected during cruise MSM49 revealed the highest fish species and cephalopod diversity. A reason for this could be that squid from MSM49 were larger and shifted their diet to squids and larger fishes: also during this cruise most squid were collected and analyzed (n = 57) potentially leading to bias when compared to cruises with lower sample size. Different myctophid species dominated the cephalopod stomach contents sampled during all research cruises, which demonstrates that myctophids are the main prey of *Sthenoteuthis pteropus*, regardless of season and location. However, squid can store hard parts of past prey items over weeks and months in their stomachs, and therefore stomach contents represent several meals and lead to bias in spatial and temporal comparisons of diet. Jobling & Breiby (1986) discovered that squid gastric fluids are only mildly acidic and otoliths immersed for two days at 4°C showed no evidence of erosion. It is suggested that hard parts are frequently evacuated from the stomach rather than digested completely, since in jumbo squid otoliths were commonly observed in the rectum (Markaida & Sosa-Nishizaki 2003).

The stomach fullness indices of the three research cruises differed slightly. Squid from cruises MSM49 and M116 mainly showed stomachs that were half full or full whereas stomachs of the cruise M119 were mainly less than half full. It is not clear whether these differences derive from different seasons, locations of capture or from the smaller sample size during M119. Cruise MSM49 had the highest frequency of occurrence of empty stomachs, but also the largest sample size. This explains the high variation in stomach fullness indices.

4.3 Primary or secondary prey?

Large amounts of copepods were found in the stomachs of squid from the cruise M116. These copepods cannot be transitory prey, since the individuals were not digested at all and there was no evidence for the presence of fish or crustaceans in the stomachs that primarily consumed them. The prey spectrum of juvenile *Sthenoteuthis pteropus* (3-15 cm ML) is dominated by meso- and macroplanktonic animals, mainly copepods, amphipods and euphausiids (Zuyev et al. 2002). This spectrum is considered to shift during ontogeny to micronektonic (>15 cm ML) and nektonic fishes and squids (>35 cm ML) (Zuyev et al. 2002). In this study only squid smaller than 25 cm mantle length contained considerable amounts of copepods in the stomachs. Copepods were almost exclusively found in squid captured

during cruise M116 taking place between May and June. Potentially a zooplankton bloom occurred during this time leading to high abundances of copepods. NASA-satellite data on chlorophyll *a* concentrations suggests that there has been a phytoplankton bloom from April to May with variant chlorophyll *a* concentrations east and southeast from Cape Verde along the African coast. Unfortunately, intense cloud cover made it difficult to analyze the chlorophyll *a* concentration in the open ocean south of Cape Verde. The hypothesis of juvenile squid (15 – 25 cm mantle length) preying on copepods seems to be very unlikely since no study has observed this behavior before and its hunting techniques seem to be inappropriate and ineffective to catch such small organisms.

However, another reason for large amounts of copepods in squid stomachs could be that the squid may have fed on gelatinous zooplankton that had ingested copepods or that is naturally associated with copepods (Ho 2001, Gasca et al. 2007). Gelatinous zooplankton is digested rapidly, even in the process of freezing the stomachs after capture, and its direct importance as prey may be largely underestimated (Piontkovski et al. 2003, Choy et al. 2016).

In the Mediterranean Sea, apex predators such as bluefin tuna (*Thunnus thynnus*), spearfish (*Tetrapturus* belone) and swordfish (*Xiphias gladius*) have been found to massively feed on gelatinous zooplankton (Cardona et al. 2012) as well as alepocephalids (slickheads) and smooth oreos occurring off southern Tasmania (Bulman et al. 2002). Other pelagic fish species may occasionally consume gelatinous zooplankton (Purcell 2001). In the eastern tropical Atlantic gelatinous zooplankton like pyrosomes are actively consumed and its abundance is therefore also controlled by squids (Piontkovski et al. 2003). Since gelatinous zooplankton can reach high abundances in open ocean upwelling areas, it plays an important role in energy and matter transformation. There is evidence, that predators such as *S. pteropus* feed on gelatinous zooplankton which represents a valuable food resource (Brodeur et al. 1987, Brodeur 1992, Arai 2005).

4.4 General diet during the ontogeny of *Sthenoteuthis pteropus*

Research Question 3: Does prey composition of Sthenoteuthis pteropus *shift during ontogeny?*

A dietary shift during ontogeny from crustacean dominated prey to fish and cephalopod dominated prey as hypothesized in the introduction cannot be generalized from the present data. The first dietary shift from crustaceans to micronektonic fishes happens in squid with a mantle length of 3 to 10 cm (Arkhipkin & Mikheev 1992). The smallest squid that could be investigated in the present study had a mantle length of 15 cm, therefore not present in the range of the first dietary shift. The second dietary shift, occurring in squid larger than 35 cm from micronektonic fishes to nektonic fishes and squids, cannot be seen either probably because of the small sample size of squid larger than 40 cm. The results of the stomach content investigations stress an opportunistic and variable feeding behavior of juvenile and adult *Sthenoteuthis pteropus* preying mainly on myctophids, but also on cephalopods and crustaceans.

4.5 Intraspecific structure

Our results suggest that *Sthenoteuthis pteropus* caught in the northern region (> 12°N) of the eastern tropical Atlantic may belong to the large late-maturing form and squid caught in the equatorial region (< 12°N) may belong the small early-maturing form (Zuyev et al. 2002). Only two mature females were caught in the northern region, but they were significantly larger than the mature females caught in the equatorial region. Additionally, all squid in the northern region were significantly larger (by 3 cm) than the squid in the equatorial region. Mature males collected in this study did not differ significantly in mantle length, but mantle length of mature males in the small and large form also overlap to a high degree, making it difficult or even impossible to distinguish those forms just by male individuals.

However, further research is needed to unravel the population structure of this species by genetic analysis since these animals are highly migratory and isolation between separate groups may be broken seasonally due to variability in hydrology and dynamics of the tropical Atlantic (Zuyev & Nikolsky 1993).

4.6 Stable isotope analysis

Research Question 4: Does Sthenoteuthis pteropus *exhibit ontogenetic shifts and intraspecific variation in trophic position and foraging area?*

4.6.1 Stable isotope analysis of muscle tissue

Stable isotope analysis of muscle tissue, the integrated trophic position of the last two months, has been used successfully for studying the trophic role of squids in food webs (Hobson et al. 1994; Takai et al. 2000; Cherel and Hobson 2005; Ruiz-Cooley et al. 2006; Stowasser et al. 2006; Cherel et al. 2009; Hunsicker et al. 2010).

The $\partial^{15}N$ stable isotope values depicts the animal's trophic position. $\partial^{15}N$ values of the muscle tissue of *Sthenoteuthis pteropus* showed an increase in trophic position by around 3‰ as the squid grows till a mantle length of 40 cm which is equivalent to a shift by one trophic level (Minagawa & Wada 1984). Therefore, larger squid occupied a higher trophic level than smaller squid indicating a shift in diet during ontogeny. These findings are in accordance with several other studies (Ruiz-Cooley et al. 2004, Parry 2008, Cherel et al. 2009, Hunsicker et al. 2010) revealing an increase by at least one trophic level with ontogeny in e.g. *Dosidicus gigas*, *Ommastrephes bartramii*, *Todarodes filippovae* and *Berryteuthis magister* by ~4, >5, ~3 and ~4‰, respectively (Parry 2008, Cherel et al. 2009, Hunsicker et al. 2010, Ruiz-Cooley et al. 2010). Interestingly, in squid larger than 40 cm $\partial^{15}N$ in muscle tissue seems to reach a plateau and stays constant or even decreases, assuming a constant trophic position with no or a negative shift in adult, large-sized squid. This may indicate that juvenile squid constantly increase its trophic position and large squid, when reached a certain mantle length, stay at one trophic position for the rest of its life or even opportunistically feed on prey of lower trophic levels when available. However, these findings are only based on three individuals and more data of larger squid are needed.

The largest female (Individual C; 47.5 cm ML) investigated in this study had 1 to 2‰ lower $\partial^{15}N$ isotopic values in muscle tissue than the other four large female individuals (> 40 cm ML). Its trophic position seems to be similar to squid smaller than 30 cm. Stable isotope data from muscle would have lead us to incorrectly assume that Individual C occupies the lowest trophic position compared with the other four large individuals. However, gladii data showed that $\partial^{15}N$ values of all squid were different throughout their life and Individual C hatched in a region with the lowest $\partial^{15}N$ baseline of all large individuals investigated. This

finding supports the opportunistic feeding behavior of *S. pteropus* preying on available prey and growing up in different areas of the tropical Atlantic. Furthermore, this finding stresses that several methods are needed, as done in this study, to assess the whole picture of the squid's ecology.

Female squid showed $\partial^{15}N$ values that were in average 1‰ higher than in male squid. This can be traced back to the fact that male squid of the species *Sthenoteuthis pteropus* are generally smaller than females (Zuyev et al. 2002, Arkhipkin & Mikheev 1992). The reasons for this are explained in section 4.1 of this study.
$\partial^{13}C$ of the muscle tissue did not show any trend and was not correlated with size, location of capture or maturity stage indicating several different migration patterns of individuals. It was expected that $\partial^{13}C$ does not correlate with size since $\partial^{13}C$ values only vary slightly with each trophic position (DeNiro & Epstein 1978, Tieszen & Slade 1983), these findings are also consistent with other studies (Cherel et al. 2009, Hunsicker et al. 2010). Female and male squid also showed no significant difference in $\partial^{13}C$ indicating that migration patterns are not sex specific.

4.6.2 Stable isotope analysis of squid gladii

Stable isotope analysis of squid gladii provides a complete picture of the trophic position and foraging habitats of individuals during ontogeny.
The gladius stable isotope values exhibit strong intra- and inter-individual variation over time and body size as well as an overall increase in trophic position and shift in habitat when the squid exceed 30 cm of mantle length. The $\partial^{13}C$ values of the most recent gladius increments of the five large squid (A, B, C, D and E) were similar (-16.0, -16.1, -16.0, -16.0, -15.5, respectively) suggesting foraging in the same habitat before they were caught. However, during their lifetime $\partial^{13}C$ values largely fluctuated in all individuals. $\partial^{13}C$ variations of 1‰ were interpreted as a habitat shift and more constant sections were assumed to reflect more stationary periods or limited movements. All individuals showed different periods of stationary feeding behavior and migration, but started to migrate when larger than 30 cm and seemed to forage in the same area when larger than 40 cm.
Sthenoteuthis pteropus spawns in the eastern equatorial Atlantic (Fig. 17) and its paralarvae are quickly dispersed in the equatorial zone. Females from the northern population

(northern of equator) migrate about 2500 km during summer from the Cape Verde Islands up to Madeira and back. The geographical range of males is slightly broader than that of females (Zuyev & Nikolsky 1993). Furthermore, immature and mature females form several large-scale groups in different geographical ranges with immature females occupying colder waters and mature females inhabiting warmer waters. Zuyev and Nikolsky (1993) identified two distinct groups of mature females in the sampling region of this study: an equatorial and a northeastern group (Fig. 17). From December until May these two groups fuse and in June until November they separate again. (Zuyev & Nikolsky 1993).

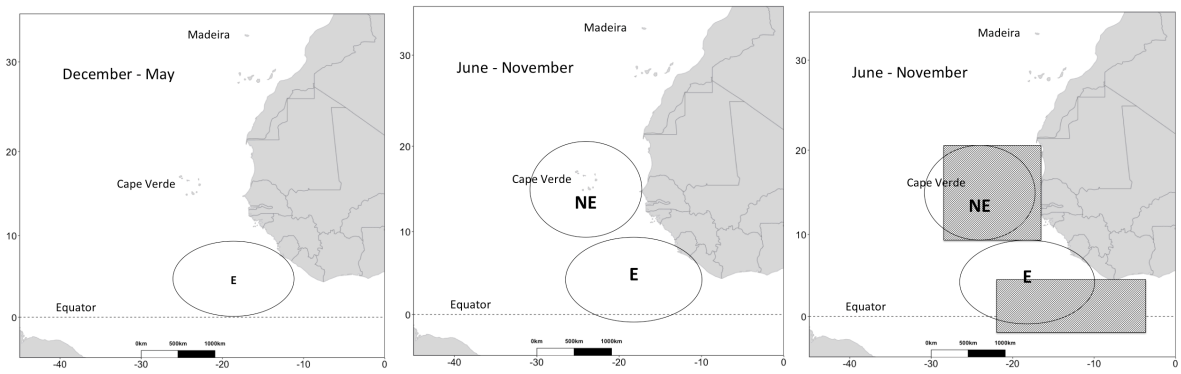

Figure 17. left & middle: Distribution of mature females of *Sthenoteuthis pteropus* in December – May and June – November in the eastern tropical Atlantic. E = equatorial group, NE = northeastern group; right: shaded area = spawning areas for the different groups of *Sthenoteuthis pteropus* in June – November in the eastern tropical Atlantic. (modified from Zuyev & Nikolsky 1993)

These differences in migration behavior would lead to differences in stable isotope values (Fig. 18). Squid that consume different prey species show varying $\partial^{15}N$ values. Different foraging areas are reflected by varying $\partial^{13}C$ values. However, despite their different life histories all squid analyzed seemed to migrate into the same area for spawning when they reached a mantle length of 30 cm. This is shown in the increased $\partial^{13}C$ values.

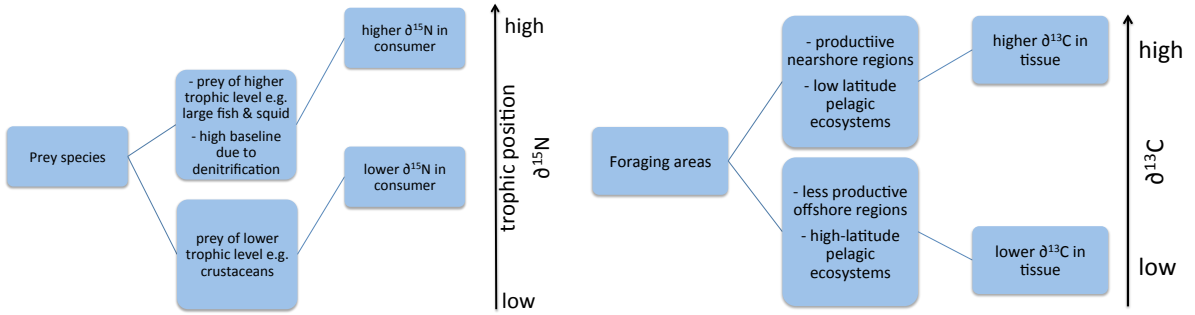

Figure 18. Factors leading to variations in ∂^{15}N (left) and ∂^{13}C (right) stable isotope values in consumers

In the gladii a continuously increasing of isotopic values throughout the squid's entire life like it occurs in other squid species, e.g. in beak and muscle tissue of *D. gigas* (Ruiz-Cooley et al. 2004, 2006), was not recognized. However, an increase in ∂^{15}N became visible when the squid exceeded 20 - 30 cm mantle length. These findings are consistent with the increasing ∂^{15}N in muscle tissue measured in squid between 15.0 to 47.5 cm mantle lengths and the second dietary shift of Zuyev et al. (2002) and Arkhipkin & Mikheev (1992) seen in squid at 35 cm mantle length (Fig.2). However, variation in ∂^{15}N can only be interpreted as a shift in trophic position when the squid does not change its foraging area (no change in ∂^{13}C), because a change in foraging area can shift the ∂^{15}N baseline (Graham et al. 2010). The squid of the present study forage in a pronounced oxygen minimum zone (OMZ) and undergo intense vertical migration (Zuyev et al. 2002). In the absence of oxygen, bacteria use nitrate to respire organic matter, this process is called denitrification. Denitrification preferentially removes ^{15}N-depleted NO_3^- and leaves residual nitrate ^{15}N-enriched (Voss et al. 2001), which leads to an increase in the baseline ∂^{15}N (Sigman et al. 1999, Graham et al. 2010). Additionally, ∂^{15}N values of marine predators are affected by vertical migration. Predators feeding on mesopelagic prey resources have higher ∂^{15}N values than predators feeding on epipelagic prey (Ménard et al. 2007, Lorrain et al. 2009) possibly as an effect of nutrient cycling (Graham et al. 2007). Therefore ∂^{15}N variation can only be defined as a shift in trophic position during a stationary period, a constant section in ∂^{13}C. In this case, the increase in ∂^{15}N is accompanied by a migration period (increase in ∂^{13}C). Without having a baseline, we cannot clearly distinguish between an increase in trophic position or an increase in ∂^{15}N baseline values due to horizontal or vertical migration. However, a baseline could be obtained by measuring stable isotope values in gelatinous zooplankton such as

tunicates or copepods at the sampling sites of the squid. Unfortunately, this data is not available yet, but would contribute to a more comprehensive picture.

Taking the muscle $\partial^{15}N$ values of individual B, D and E into account, it can be seen that they occupy the highest trophic level compared with smaller specimens (B: 12.8‰, D: 12.1‰, E: 13.3‰). This could underpin the hypothesis of an increase in trophic position seen in gladius with size.

It was also observed that variable isotopic shifts occur along the proostracum. This is in accordance with previous studies of *Dosidicus gigas* and *Berryteuthis magister* investigating variable $\partial^{15}N$ and $\partial^{13}C$ values along the gladius and strong intra-individual variation (Hunsicker et al. 2010, Lorrain et al. 2011, Ruiz-Cooley et al. 2013), revealing a complex life history of these squids. Overlapping $\partial^{13}C$ and $\partial^{15}N$ values were observed among some individuals, indicating foraging in the same habitat and feeding at the same trophic level, but every squid reveals a unique isotopic pattern through its life. The differences in $\partial^{13}C$ and $\partial^{15}N$ in gladii throughout almost all their life suggests that they foraged in different habitats with different isotopic baselines. Simultaneous analysis of muscle tissue and gladii revealed no relationship between muscle tissue and the most recent gladii $\partial^{15}N$ and $\partial^{13}C$ values. $\partial^{15}N$ values in gladii were incessantly lower (3.9 $^{+}/_{-}$ 0.8‰) compared to muscle tissue and revealed a smaller ontogenetic shift in isotope values. This could also be seen in *Dosidicus gigas* (Ruiz-Cooley et al. 2006, 2010, Cherel et al. 2009) and *Berryteuthis magister* (Hunsicker et al. 2010). These findings make sense keeping in mind that gladii consist mainly of chitin and chitin is depleted in ^{15}N but not in ^{13}C relative to mantle tissue (DeNiro & Epstein 1981, Webb et al. 1998). The $\partial^{13}C$ values of muscle and gladii tissue were significantly different (Welch two sample t-test, $p = 0.04$), but in a narrow range (0.6 +/- 0.5‰). It has to be kept in mind that gladius and muscle have different turnover rates. Muscle tissue integrates the trophic position and foraging habitat of up to the last two months. The gladii reflects shorter time periods (Arkhipkin & Mikheev 1992), revealing finer temporal variation in isotopic values that were not present in muscle tissue isotopic values. In *Sthenoteuthis oulaniensis* daily mean growth increments were 0.77, 0.79 and 1.25 mm between 30 and 270 mm gladius length of males, medium-sized and large-sized females respectively (Bizikov 1991). This means that an increase of 10 or 20 mm of gladius increments correspond to 8 to 25 days of the squid's life. However, it should be considered that gladius growth can be

influenced by temperature, food availability and ontogenetic stage of the squid (Perez & O'Dor 2000).

The knowledge of the differences between muscle tissue and gladii isotopic values can be useful for comparison in future studies when only one tissue is available. The differences in muscle and gladii isotopic values may be due to individual changes in feeding strategies, prey availability or temporal and spatial differences in prey isotopic values that are only visible in techniques that unravel short-term changes, in this case with gladii isotopic values. Traditionally, conspecific individuals have been evaluated as ecologically equivalent, although several studies stress that individual specialization varies widely among species and populations (Lorrain et al. 2011). Individual specialization may have been underestimated in the past and may have potentially important ecological, evolutionary and conservation implications (Bolnick et al. 2003, Bearhop et al. 2004). Phenotypic differences among individuals, individual specialization and spatial or temporal variation in prey availability may enhance the separation into different foraging specialists (Estes et al. 2003) as seen in the present study. Its complex life history strategy reveals the high plasticity and capability of *Sthenoteuthis pteropus* to rapidly colonize new areas and niches made available and to adapt to new environmental conditions.

4.7 The current trophic position of *Sthenoteuthis pteropus* compared to the study of Zuyev et al. (2002)

Research question 5: How is the current trophic position of Sthenoteuthis pteropus *compared to the study of Zuyev et al. (2002)?*

Specimens of *Sthenoteuthis pteropus* that are smaller than 30 cm seemed to regularly feed on high densities of fishes of the genus *Vinciguerria* (family: Phosychthidae). In the equatorial area of the Atlantic Ocean *Vinciguerria nimbaria* and *Vinciguerria attenuata* are small mesopelagic fish that form loose schools that occur in clusters during the daytime and form large aggregations during the night (Ménard & Marchal 2003). *Vinciguerria nimbaria* is an essential prey species for tuna in the Atlantic Ocean (Marchal & Lebourges 1996, Menard et al. 2000, Ménard & Marchal 2003, Champalbert et al. 2008). Ménard and Marchal (2003) investigated that a single school of *V. nimbaria* (n = 24 400) is able to feed a single tuna school for two months. In the study of Zuyev et al. (2002) the genus Phosychthidae are not

listed as abundant prey in the diet of *Sthenoteuthis pteropus*, however in the present study they were found in high abundance in some squid stomachs. As mentioned above, tuna which is overfished in the Atlantic Ocean heavily feeds on *V. nimbaria*. The results of the present study could be the first evidence of *S. pteropus* taking over the niche of a large predatory fish by switching to its main prey resource. However, this statement has to be treated with caution, because the data of Zuyev et al. (2002) on the diet of *S. pteropus* is not published in detail.

Nevertheless, the trophic position of *Sthenoteuthis pteropus* is as Zuyev et al. (2002) suggested. The food species spectrum of *S. pteropus* potentially changes during ontogeny from lower to higher trophic levels.

6 Conclusion

In this study three different methods that complemented one another (stomach content analysis, analysis of muscle tissue stable isotopes, analysis of gladius stable isotopes) are combined to investigate the general diet, trophic position and foraging areas of *Sthenoteuthis pteropus* in the tropical eastern Atlantic and to uncover individual specialization and changes in feeding and migration. The following research questions were answered:

1 What is the ecological role and general diet of *Sthenoteuthis pteropus* in the food web of the eastern tropical Atlantic?

The ommastrephid squid *Sthenoteuthis pteropus* is an abundant key species in the eastern tropical Atlantic food web. It preys mainly on myctophids, an important family of small pelagic fishes of lower trophic level, but also on cephalopods and crustaceans. Furthermore, *S. pteropus* probably competes with larger predatory fishes for the same prey species. It is an opportunistic carnivore that may also feed on gelatinous zooplankton, and shows high intra- and inter-individual variation in trophic position, feeding strategy and migration behavior. Its complex life history explains the high plasticity of *S. pteropus* and its high potential to rapidly colonize new areas and to easily adapt to environmental variability.

2 Are there differences in prey composition between three research cruises?

There are no significant differences in prey composition of *Sthenoteuthis pteropus* between the three research cruises. The results support that myctophids are the main prey of this squid species regardless of geographical distribution or sampling season.

3 Does prey composition of *Sthenoteuthis pteropus* shift during ontogeny?

A shift in the general diet during ontogeny in *Sthenoteuthis pteropus* could not be seen, probably due to the small specimen sample of large-sized squid. The results also support the opportunistic feeding behavior of *S. pteropus* on available prey, especially myctophids.

4 Does *Sthenoteuthis pteropus* exhibit ontogenetic shifts and intraspecific variation in trophic position and foraging area?

The stable isotope values of mantle tissue showed an ontogenetic shift in trophic position by one trophic level as the squid grows, but no visible trend in foraging area. Gladii stable isotope values showed variable trophic positions and foraging areas during ontogeny especially during the first ontogenetic stages (1 – 20 cm mantle length). When reaching a mantle length of 30 cm, all individuals seemed to migrate into the same foraging habitat and potentially increased their trophic position. However, the increase in $\partial^{15}N$ could also be due to intense denitrification in the foraging habitat that would lead to a baseline shift.

Without gladii stable isotope values we would have assumed that the largest individual of 47.5 cm mantle length inhabits the same trophic level as squid of 30 cm mantle length. However, gladii isotopic values revealed, that the largest individual had a lower isotopic baseline throughout its entire life possibly leading to lower $\partial^{15}N$ values in muscle tissue.

5 How is the current trophic position of *Sthenoteuthis pteropus* compared to the study of Zuyev et al. (2002)?

The study of Zuyev et al. (2002) on the trophic ecology of *Sthenoteuthis pteropus* trophic ecology is exclusively based on stomach content analysis. The present study could confirm their findings that the food species spectrum of this squid most likely changes during ontogeny. By using modern stable isotope techniques to investigate the trophic ecology of squid a high intra- and inter-individual variation in foraging strategies and areas was documented. Potentially, this study shows first evidence of *S. pteropus* switching its diet to new prey species that are released from high competition pressure by other large predatory fishes.

Stable isotope analysis of the gladii was capable of depicting fine spatio-temporal variation during ontogeny in individual foraging strategies of *Sthenoteuthis pteropus*. It compensates bias in stomach content analysis and stable isotopes on metabolically active tissue. The combination of the three different analyses provides a broad and profound picture of the trophic ecology of *S. pteropus* and helps us to understand how anthropogenic and natural impacts can impact feeding strategies.

In future studies, it should be crucial to analyze squid isotope values and diet from multiple years and different seasons to better document temporal variation. Comparisons between isotopic values of different hard tissues such as eye lenses, beaks, gladii and soft tissue such as muscle tissue of the same individuals should be performed to elucidate the variation of isotopic signals from different tissues and to enable the comparison of different squid isotope studies. Combining stable isotopes measurements with tagging studies would be auspicious to investigate migration patterns simultaneously with changes in trophic position.

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Finally, a big thank to my friends, family and boyfriend who have supported me throughout the thesis and my studies with unfailing support, continuous encouragement and never ending humour.

7 Appendix

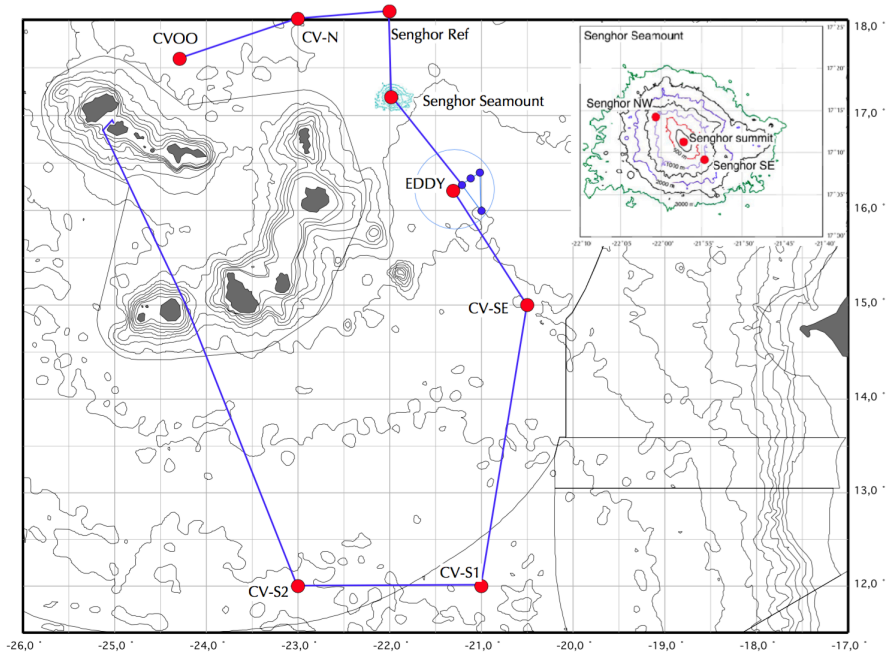

Figure 19. MSM49 study sites and cruise track taking place in November/December 2015. Inset: Senghor Seamount stations

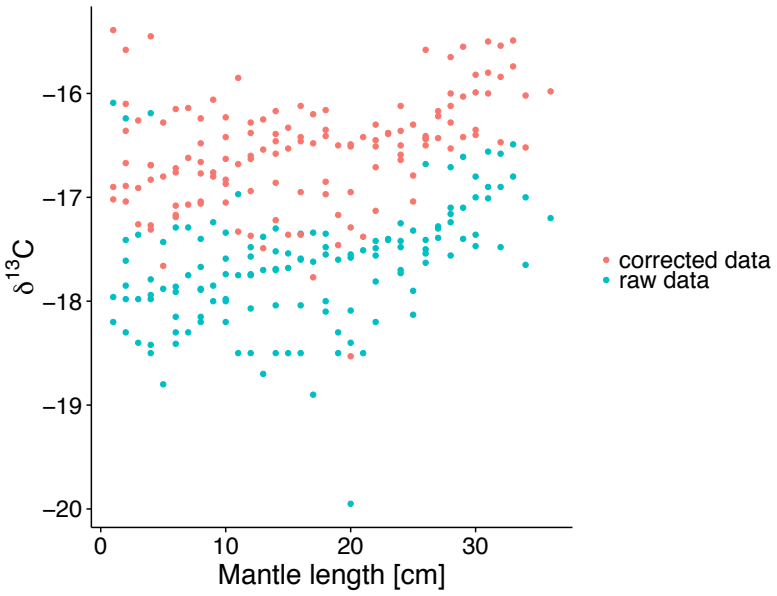

Figure 20. Corrected and raw ð13C data of 129 Sthenoteuthis pteropus caught in the tropical eastern Atlantic in 2015

Table 7. Summary of the catches of *Sthenoteuthis pteropus* during the three research cruises in 2015 in the eastern tropical Atlantic (Unknown individual from M116 excluded from analysis)

Cruise	Location	Date	Female	Male	Immature	Total	ML (cm)	Sum per Cruise
MSM49	CVOO	02.12.15	6			6	21.5 - 30.5	
	Senghor Ref	04.12.15	3	1		4	21.0 - 45.5	
	Senghor NW	06.12.15	4	6		10	18.4 - 34.6	
	Senghor Summit	07.12.15	2	2		4	19.7 - 41.0	
	Senghor Slope SE	10.12.15	3			3	21.5 - 47.5	
	Eddy Core	11.12.15	13	1		14	18.8 - 43.4	
	CVSE	14.12.15	3			3	26.3 - 36.8	
	CVS1	16.12.15	7	1		8	18.9 - 32.0	
	CVS2	18.12.15	4	1		5	18.7 - 30.8	57
M116	8°N 23°W	May/June	4	2		6	15.2 - 27.2	
	9° N 21 - 40° W	May/June	2			2	21.1 - 27.9	
	10° N 28 °W	May/June	3	1		4	17.9 - 33.5	
	10°N 25° W	May/June	5	4		9	15.6 - 39.0	
	10° N 35° W	May/June	1	1		2	17.1 - 25.3	
	11°N 19°W	May/June	5			5	20.4 - 25.7	
	12°N 23° W	May/June	10	5	1	16	15.5 - 33.1	
	12°N 25°W	May/June	5	1		6	17.9 - 30.5	
	unknown	May/June	1			1	22,3	51
M119	11° N 21° W	Sept./Oct.	3	1		4	19.5 - 25.0	
	2°N 23°W	Sept./Oct.	14	4		18	13.1 - 31.0	22
Total			**98**	**31**	**1**	**129**	**18.4 - 47.5**	122

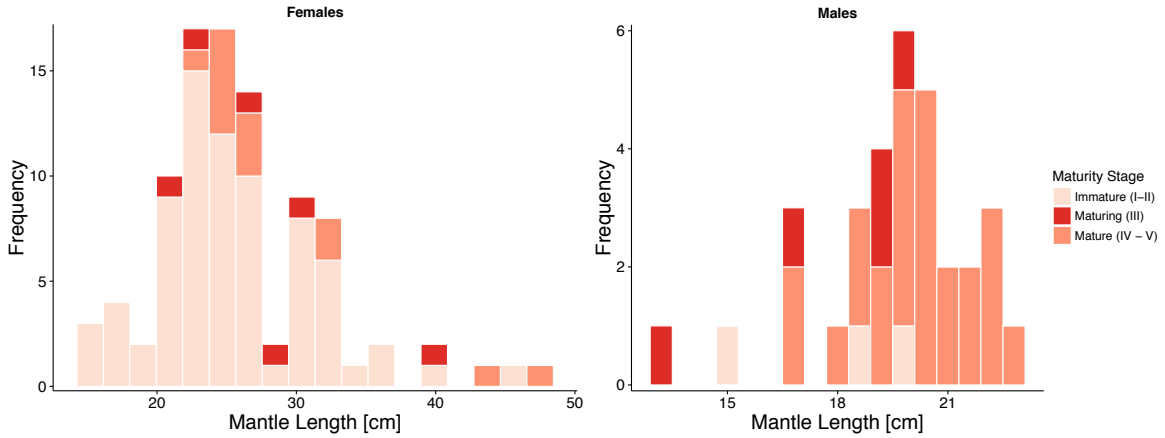

Figure 21. Frequency of the maturity stages of all *Sthenoteuthis pteropus* caught in 2015 in the eastern tropical Atlantic (n =129).

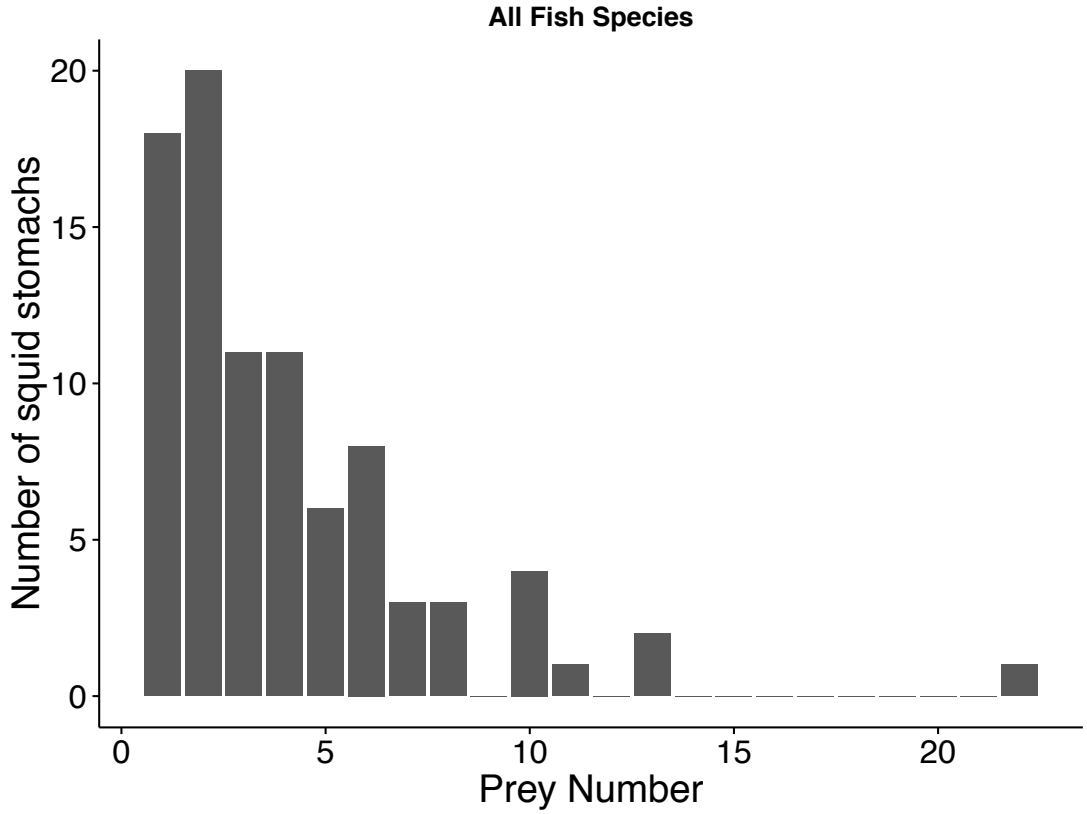

Figure 22. Frequency distribution of the number of fish species found in the stomachs of *Sthenoteuthis pteropus* caught in the eastern tropical Atlantic in 2015

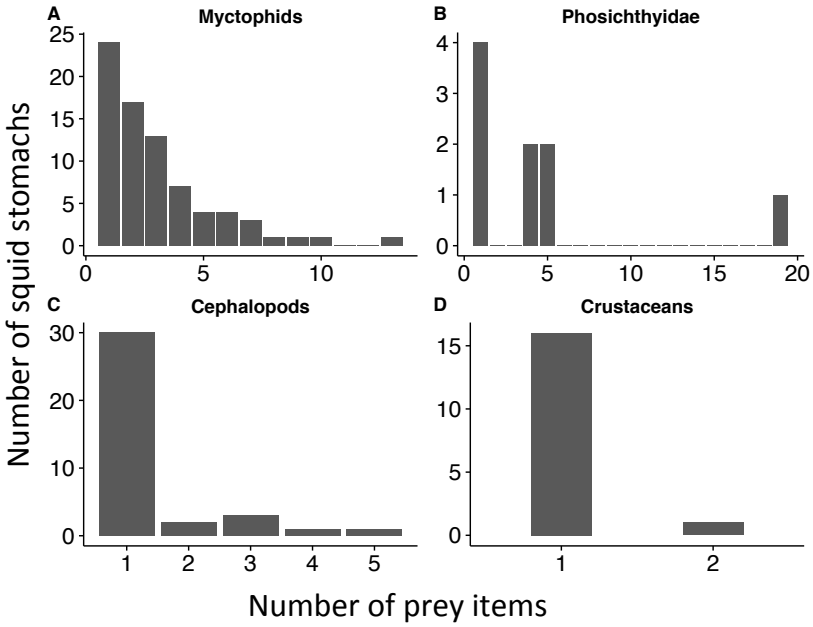

Figure 23. Frequency distributions of the number of prey items found in *Sthenoteuthis pteropus* stomachs. A = Myctophids, B = Phosichthydae, C = Cephalopods, D = Crustaceans

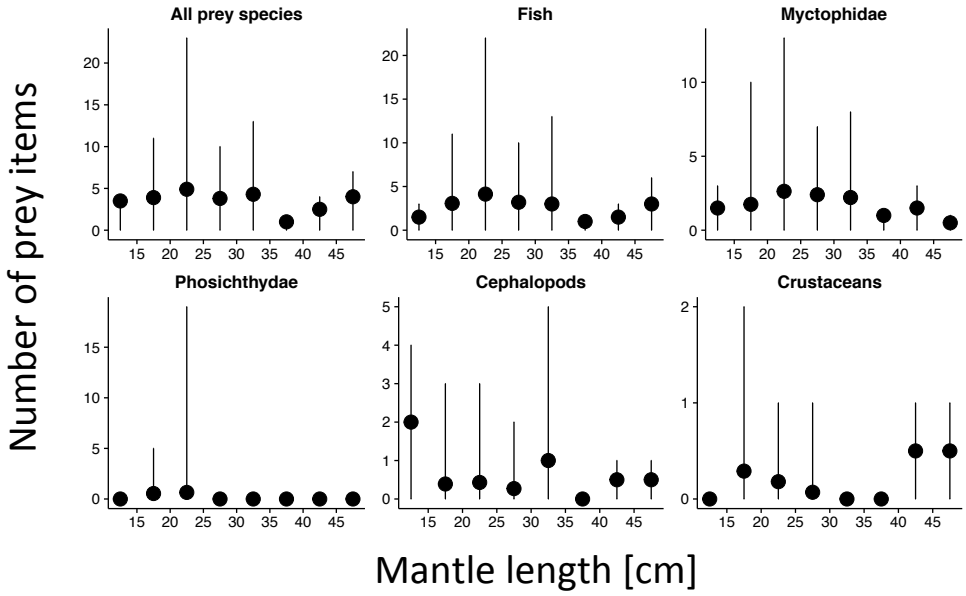

Figure 24. Variability of the average and range in number of the most important prey items of *Sthenoteuthis pteropus* from the eastern tropical Atlantic for 5 cm size intervals; Crustaceans includes euphausiids and decapods; sample size per size interval: 10 – 14 cm = 1; 15 – 19 cm = 25; 20 – 24 cm = 49; 25 – 29 cm = 30; 30 – 34 cm = 15; 35 – 39 cm = 5; 40 – 44 cm = 1, 45 – 50 cm = 3

Table 8. SIMPER Analysis of MSM49 and M116

	MSM49	M116			
Species	**Av.Abund**	**Av.Abund**	**Av.Diss**	**Contrib%**	**Cum.%**
Hygophum spp.	0	1.73	1.73	4.9	4.9
Hygophum reinhardti	0	1.73	1.73	4.9	9.81
Hygophum proximum	0	1.41	1.41	4	13.81
Myctophum spinosum	1.41	0	1.41	4	17.82
Symbolophorus	0	1.41	1.41	4	21.82
Notoscopelus caudispinosus	0	1.32	1.31	3.73	25.55
Symbolophorus rufinus	0	1.32	1.31	3.73	29.27
Bathylagidae spp.	1.32	0	1.31	3.73	33
Diaphus fragilis	0	1.19	1.19	3.37	36.37
Exocoetius obtusirostris	1.19	0	1.19	3.37	39.73
Lestidiops sphyrenoides	1.19	0	1.19	3.37	43.1
Chauliodus sloani	1.19	0	1.19	3.37	46.47
Cubiceps pauciradiatus	1.19	0	1.19	3.37	49.84
Cirrate octopods	1.19	0	1.19	3.37	53.2
Diaphus	0	1	1	2.83	56.03
Lampanyctus intracarius	1	0	1	2.83	58.87
Lampanyctus nobilis	0	1	1	2.83	61.7
Lepidophanus gaussi?	0	1	1	2.83	64.53
Bregmacerotidae	0	1	1	2.83	67.36
Paralepidae	0	1	1	2.83	70.19
Lestrolepis intermedia	1	0	1	2.83	73.02
Mastigoteuthidae	1	0	1	2.83	75.85
Histioteuthidae	1	0	1	2.83	78.68
Onychoteuthidae	1	0	1	2.83	81.52
Vinciguerria attenuata	2.09	1.19	0.9	2.54	84.06
Diaphus dumerilii	1.78	1	0.78	2.2	86.26
Myctophum spp.	1.19	1.93	0.74	2.11	88.37
Hygophum macrochir	1	1.68	0.68	1.93	90.3

Table 9. SIMPER- Analysis of MSM49 and M119

	MSM49	M119			
Species	**Av.Abund**	**Av.Abund**	**Av.Diss**	**Contrib%**	**Cum.%**
Vinciguerria attenuata	2.09	0	2.56	5.3	5.3
Decapoda	1.68	0	2.06	4.27	9.57
Myctophum spinosum	1.41	0	1.73	3.59	13.16
Lampanyctus spp.	1.32	0	1.61	3.34	16.5
Merluccius sp.	1.32	0	1.61	3.34	19.84

Gonichthys sp.	1.19	0	1.46	3.02	22.86
Myctophum spp.	1.19	0	1.46	3.02	25.87
Myctophum selenops	1.19	0	1.46	3.02	28.89
Symbolophorus rufinus	0	1.19	1.46	3.02	31.91
Lestidiops sphyrenoides	1.19	0	1.46	3.02	34.93
Chauliodus sloani	1.19	0	1.46	3.02	37.95
Cubiceps pauciradiatus	1.19	0	1.46	3.02	40.97
Ommastrephidae	0	1.19	1.46	3.02	43.98
cirrate octopods	1.19	0	1.46	3.02	47
Diogenichthys atlanticus	0	1	1.22	2.54	49.54
Diaphus lucidus	0	1	1.22	2.54	52.08
Hygophum macrochir	1	0	1.22	2.54	54.62
Lampanyctus intracarius	1	0	1.22	2.54	57.16
Lampanyctu festivus	0	1	1.22	2.54	59.69
Lampanyctus nobilis	0	1	1.22	2.54	62.23
Lepidophanes guentheri	0	1	1.22	2.54	64.77
Myctophum obtusirostre	0	1	1.22	2.54	67.31
Symbolophorus sp.	0	1	1.22	2.54	69.85
Lestrolepis intermedia	1	0	1.22	2.54	72.38
Bolitinidae	1	0	1.22	2.54	74.92
Pyroteuthidae	0	1	1.22	2.54	77.46
Mastigoteuthidae	1	0	1.22	2.54	80
Histioteuthidae	1	0	1.22	2.54	82.54
Onychoteuthidae	1	0	1.22	2.54	85.07
Euphausiid	1	0	1.22	2.54	87.61
Diaphus dumerilii	1.78	1	0.95	1.98	89.59
Ceratoscopelus warmingii	1	1.63	0.77	1.59	91.18

Table 10. SIMPER-Analysis of M116 and M119

	M116	**M119**			
Species	**Av.Abund**	**Av.Abund**	**Av.Diss**	**Contrib%**	**Cum.%**
Myctophum spp.	1.93	0	2.28	5.02	5.02
Hygophum spp.	1.73	0	2.04	4.5	9.52
Hygophum reinhardti	1.73	0	2.04	4.5	14.02
Hygophum macrochir	1.68	0	1.98	4.37	18.39
Decapoda	1.63	0	1.92	4.22	22.61
Hygophum proximum	1.41	0	1.67	3.67	26.28
Notoscopelus caudispinosus	1.32	0	1.55	3.42	29.7
Diaphus fragilis	1.19	0	1.4	3.09	32.79
Gonichthys sp.	1.19	0	1.4	3.09	35.88

Myctophum selenops	1.19	0	1.4	3.09	38.97
Vinciguerria attenuata	1.19	0	1.4	3.09	42.05
Ommastrephidae	0	1.19	1.4	3.09	45.14
Euphausiid	1.19	0	1.4	3.09	48.23
Diogenichthys atlanticus	0	1	1.18	2.6	50.83
Diaphus spp.	1	0	1.18	2.6	53.42
Diaphus lucidus	0	1	1.18	2.6	56.02
Lampanyctu festivus	0	1	1.18	2.6	58.62
Lampanyctus spp.	1	0	1.18	2.6	61.21
Lepidophanus gaussi?	1	0	1.18	2.6	63.81
Lepidophanes guentheri	0	1	1.18	2.6	66.41
Myctophum obtusirostre	0	1	1.18	2.6	69
Bathylagidae	0	1	1.18	2.6	71.6
Exocoetius obtusirostris	0	1	1.18	2.6	74.2
Bregmacerotidae	1	0	1.18	2.6	76.8
Merluccius sp.	1	0	1.18	2.6	79.39
Paralepidae	1	0	1.18	2.6	81.99
Bolitinidae	1	0	1.18	2.6	84.59
Pyroteuthidae	0	1	1.18	2.6	87.18
Vinciguerria nimbaria	1.9	1	1.06	2.33	89.52
Hygophum taaningi	1.82	1.19	0.75	1.64	91.16

Table 11. ð^{15}N, ð^{13}C and C/N of muscle tissue (n=54) of *Sthenoteuthis pteropus* caught in the eastern tropical Atlantic in 2015

Individual	ML	ð^{15}N [‰]	ð^{13}C [‰]	Sex	Mat. Stage	C/N	Station
M1	30.5	11.491	-17.156	female	1	3.61	CV00
M3	28.5	11.732	-15.513	female	1	3.21	CV00
M4	25.5	11.176	-15.717	female	1	3.22	CV00
M5	25.3	11.238	-16.85	female	1	3.32	CV00
M6	29.5	11.242	-17.286	female	1	3.57	CV00
M7	35.5	12.167	-16.865	female	1	3.51	S. Ref
M8	45.5	11.957	-16.511	female	2	3.76	S. Ref
M9	25.5	11.223	-16.97	female	1	3.49	S. Ref
M11	34.6	12.187	-16.897	female	1	3.51	S. NW
M13	22.5	11.168	-16.874	female	1	3.42	S. NW
M20	23.6	11.141	-17.095	female	1	3.52	S. NW
M21	24.3	11.581	-15.163	female	1	3.16	S. Summit
M22	22.6	10.378	-17.324	female	1	3.51	S. Summit
M25	41.0	12.077	-17.082	female	2	3.76	S. Summit
M26	21.5	10.949	-16.977	female	1	3.46	S. Slope SE

M27	40.5	12.78	-15.998	female	2	3.39	S. Slope SE
M28	47.5	11.273	-16.871	female	5	3.67	S. Slope SE
M29	27.3	11.369	-16.341	female	2	3.39	Eddy Core
M30	18.8	10.359	-17.001	female	1	3.49	Eddy Core
M32	27.3	11.481	-16.52	female	1	3.52	Eddy Core
M33	26.8	11.901	-16.48	female	1	3.43	Eddy Core
M34	27.3	11.406	-16.328	female	1	3.41	Eddy Core
M35	23.5	11.178	-16.722	female	1	3.45	Eddy Core
M37	21.8	10.305	-17.093	female	1	3.52	Eddy Core
M38	32.2	12.05	-16.37	female	1	3.41	Eddy Core
M39	26.7	11.562	-16.354	female	1	3.44	Eddy Core
M40	43.4	13.323	-16.357	female	5	3.59	Eddy Core
M41	40.0	12.939	-14.778	female	2	3.23	Eddy Core
M43	21.1	10.22	-16.793	female	3	3.51	Eddy Core
M44	25.5	11.238	-16.204	female	4	3.49	Eddy Core
M45	23.8	10.311	-16.101	female	1	3.30	Eddy Core
M46	33.0	12.327	-16.227	female	1	3.46	Eddy Core
M49	32.6	12.206	-15.644	female	1	3.24	CVSE
M50	36.8	11.711	-15.681	female	1	3.24	CVSE
M51	30.0	12.032	-16.472	female	1	3.44	CVS1
M52	27.1	11.214	-16.649	female	1	3.42	CVS1
M53	31.4	11.751	-15.078	female	1	3.22	CVS1
M55	25.4	11.854	-16.623	female	2	3.47	CVS1
M57	32.0	11.914	-16.644	female	1	3.47	CVS1
M58	29.8	11.218	-16.278	female	1	3.45	CVS1
M59	23.4	11.218	-15.317	female	1	3.20	CVS1
M60	31.3	11.61	-16.814	female	1	3.54	CVS1
M61	30.8	10.865	-16.872	female	1	3.50	CVS2
M62	22.6	10.583	-16.798	female	1	3.43	CVS2
M10	21.0	9.676	-16.553	male	5	3.45	S. Ref
M12	19.5	10.658	-16.817	male	5	3.43	S. NW
M14	19.0	11.637	-16.874	male	3	3.52	S: NW
M16	20.5	10.394	-17.111	male	5	3.50	S. NW
M17	18.4	9.889	-15.641	male	5	3.21	S. NW
M18	19.0	10.244	-16.034	male	3	3.21	S. NW
M19	20.0	10.195	-16.207	male	1	3.29	S. NW
M23	20.4	11.264	-16.925	male	5	3.47	S. Summit
M31	19.7	10.677	-16.297	male	5	3.44	Eddy Core
M42	20.7	11.118	-15.65	male	5	3.21	Eddy Core

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9 Declaration of authorship

Herewith I certify that the present thesis, apart from the consultation of my supervisors, was independently prepared by me. No other than the indicated resources and references were used. This thesis was presented to no other place within the scope of an examination procedure. The written thesis is identical with the electronic one.

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Ich erkläre, dass ich meine Masterarbeit „The trophic ecology of the oceanic orangeback squid *Sthenoteuthis pteropus* in the eastern tropical Atlantic“ selbstständig und ohne Benutzung anderer als der angegebenen Hilfsmittel angefertigt habe und dass ich alle Stellen, die ich wörtlich oder sinngemäß aus Veröffentlichungen entnommen habe, als solche kenntlich gemacht habe. Die Arbeit hat bisher in gleicher oder ähnlicher Form oder auszugsweise noch keiner Prüfungsbehörde vorgelegen.
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